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THE GROWTH-REPRODUCTION CYCLE

By

H. L. EICHHORN

(*Received for Publication November 17, 1939*)

Research in the field of Growth has pointed out the similarity of the weight-age curves of birds and mammals and a certain persistent schedule linked with sexual change has been shown to apply. No definite connection between growth and reproduction seems to have been established altho both are conversions of the same raw material (food) into the same product (body mass) by the same "machine".

The intention now is to arrive at this connection, not by using the recorded observations of any particular living being but by analyzing the over all action of a perfect elementary living "machine". This "machine" will be considered as converting a raw material called "food" into products appearing as frame, tissue, fluids, etc. which will be referred to as "body mass". Some of this conversion takes place during growth; some during reproduction. The environment assumed is such as to require no expenditure of energy for foraging, external work or temperature adjustment. No consideration of mechanism or chemistry involved is to be attempted.

Anyone acquainted with thermodynamics will recognize, in such an attempt, some similarity to the work of Carnot (1824). The paths followed by the perfect elementary heat engine in the cycle described by Carnot cannot be traced by empirical methods. Similarly, the ideal paths of food consumption by a perfect elementary living body cannot be found by laboratory or test pen methods altho at certain stages they seem clearly indicated.

The following conditions are set up as criterions of perfection of the elementary living machine as measured by efficiency of operation.

1. All raw material (consumed food) must be accounted for in performance (growth, maintenance and reproduction) without loss. Thus food intake becomes a direct measure of performance.

2. Reproduction occurs for the purpose of continuing the species in balance with other life. Growth fits the living machine for the reproduction procedure. For highest efficiency, an inanimate machine of any description must operate at its rated capacity. It must be neither too large nor too small for the work to be done. Similarly, the reproductive activity of the most efficient elementary living machine must reach and not exceed the ability of that machine to reproduce.

3. Food intake is a measure of vital work done. In the engineering

sense, the rate of doing work is termed "power". Therefore rate of food consumption may be considered as a measure of "vital power". Where there is reproduction, there must be an interlocking chain of individuals and no individual can be studied entirely apart from forbear and offspring. In such a perfect chain, it must be shown that the vital power given up by the progenitor in reproduction is exactly equal to that acquired by offspring.

No attempt is being made to define "food". It may consist entirely of essential nutrients or certain carriers or bulk materials may be necessary constituents. In any case, it may be divided into two components in much the same sense that a force may be resolved into components along two axes. Consider the ration (food consumed per unit time) as divided into two such components. The *sustaining component* furnishes the necessary materials to maintain existing body structure and supplies the necessary energy for continued existence at a fixed level without increase or decrease in body mass and without reproduction. The *creative component* is that portion which is required in addition to the sustaining component to increase body mass by growth or by reproduction. It includes all materials, energy carriers and incidental substances which are required for increase of body mass by growth or by reproduction but which would not be required if only existence at a constant level of life and body mass were maintained.

It is doubtful whether the proportions of nutrients in the two components can be given at the present time. "It is, however, usual in physiology to consider nitrogen or protein metabolism as primarily concerned with metabolism of building materials while carbohydrates and fats supply the energy requirements." (Von Bertalanffy in Human Biology, Vol. 10, No. 2.) It follows that the protein content of the creative component must be higher than that of the sustaining component, the difference being made up largely in the sustaining component by a greater content of the energy carriers.

We will now consider (Fig. 1) the logical variation in the proportion of the two components during growth, reproduction and the periods of transition between the two. As growth, the first predominant activity after birth, proceeds, body mass increases and an increasing share of the ration must be apportioned to sustain this body mass (sustaining component) leaving less food proportionately available for further increase in body mass (creative component). Therefore the increment of the creative component decreases and reaches a momentary value of zero at the point '3'.

At '3', the value of creative component *for growth* has reached its

maximum. Further movement for growth alone would mean decrease in creative component allotment. Reproductive activity, when it occurs, will mean increase in creative component requirement up to the capacity of the machine to reproduce (Second Criterion). Therefore for efficient operation, it is necessary that the movement toward full reproductive activity begin at once to take up the creative component units now being dropped by growth and add to them additional creative component units until full reproductive activity is reached at '4'. Between '3' and '4', allotment of creative component for reproductive activity increases to its maximum while creative component allotted to growth approaches and reaches zero. It follows therefore that the increment of sustaining component decreases after '3' and becomes zero at '4'.

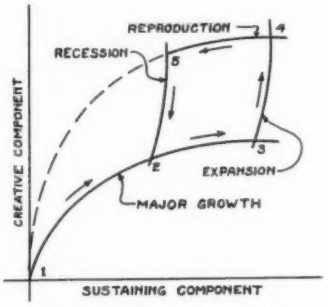

Fig. 1. Cycle paths of elementary living machine.

Growth has now served its purpose. To satisfy Second Criterion, the living machine must now be used to full capacity for reproduction. As the end of reproductive activity approaches so must also the exhaustion of the machine approach. In other words, both components must approach zero after the peak at '4' is reached. This directs the path toward the intersection of the creative and sustaining component axes (zero vital power).

Where repetition of reproductive activity is not a part of the plan, this direction is followed until all available substance in the machine is exhausted. Where a repetition of reproduction is planned, another path such as 5-2 is required to bring the machine back to the ration level for zero reproduction and zero growth. Then further growth preceding the next period of reproduction will take place along the repeated path 2-3.

Thus the relationship between growth and reproduction in the ideal living machine takes the form of a cycle. In Fig. 1 the paths have been

labeled major growth (modifying term to be explained later), expansion, reproduction and recession.

Several very useful characteristic straight lines are to be found on a creative-sustaining component or CS diagram. Lines radiating from the origin such as OA and OH on Fig. 2 are lines of constant percentage of protein of the ration (constant R-lines). Lines crossing the axes at 45 degrees (when equal scales are used at the two axes) such as UV are lines of constant food consumed in unit time (constant F-lines). Other lines parallel to each other lying at an angle dependent on the proportion between the percentage protein contents of the creative and sustaining components such as CD and EF are lines of constant weight of protein consumed per unit time (constant Q-lines). There is, in addition this important relationship: If a constant F-line such as UV is divided at equally spaced points, the constant R-lines connecting these points with the origin are themselves equally spaced with regard to percentage of protein or 'R'. In the subject matter to follow, such a 45 degree line will be called 'the line for equal R-line distribution.'

Point '4' (Fig. 1) is the peak of efficient food consumption. The sustaining component is at its maximum: therefore the element of the expansion path at '4' is vertical. The creative component is at its maximum: therefore the element of the reproduction path is horizontal. The expansion and reproduction paths may be considered as generating each other as these conditions are maintained. The expansion path is the locus of points of zero increment in creative component for such paths as reproduction and growth. The reproduction path is the locus of points of zero increment in sustaining component for such paths as expansion and recession.

On Fig. 2, let OV be the creative component axis and OU the sustaining component axis of a CS diagram. Let AB be the infinitesimal element of the expansion path at the point '4' lying between the constant R-lines OA and OH and between the constant Q-lines CD and EF. Let r/q represent the ratio of change in R and Q between the points A and B. VU is the 45 degree line for equal R-line distribution and N is the number of equal segments into which VU is divided. Let R_c and R_s be the percentage of protein contents of the creative and sustaining components respectively. Let 'c' and 's' be the number of weight units of creative and sustaining components consumed at the point 'A' in unit time.

Draw LH perpendicular to AB and AJ perpendicular to EF. Triangles ALH and VOU are similar.

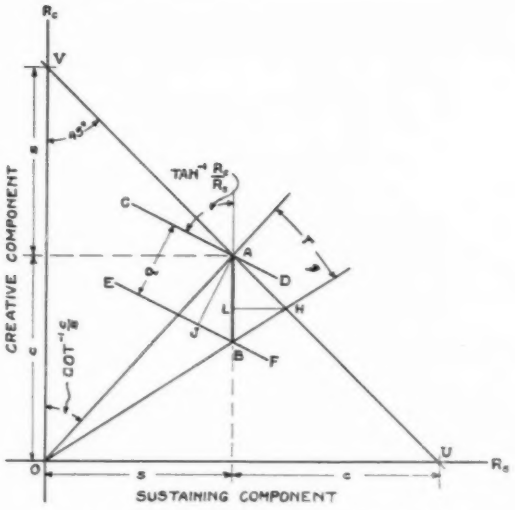

Fig. 2. Element of expansion path at point 4.

$AL/VO = AH/VU \qquad VO = (c + s) \qquad AH/VU = 1/N$

$\therefore AL = (c + s)/N \qquad LH = AL = (c + s)/N$

$\angle ABH = \angle VOH = \angle VOA + \angle AOH$ but, since AB is infinitesimal, $\angle AOH$ is infinitesimal. Therefore $\angle ABH = \angle VOA = \cot^{-1}(c/s)$

$LB = LH \cot(\cot^{-1} c/s) = (c + s)(c)/Ns$

$AB = AL + LB = (c + s)/N + (c + s)(c)/Ns = (c + s)^2/Ns$

$$AJ = AB \sin\left(\tan^{-1}\frac{R_c}{R_s}\right) = \frac{(c + s)^2}{Ns}\frac{R_c}{\sqrt{R_c^2 + R_s^2}} = \frac{R_c(c + s)^2}{Ns\sqrt{R_c^2 + R_s^2}}$$

This is the lineal value of AJ according to the CS scale. To get its value as 'q' adjusted to the difference between the CS and the protein scales, multiply AJ by $\sqrt{R_c^2 + R_s^2}$.

$q = AJ\sqrt{R_c^2 + R_s^2} = R_c(c + s)^2/Ns$

$r = (R_c - R_s)/N$

$$r/q = (R_c - R_s)/N \times Ns/R_c(c + s)^2 = \frac{s(R_c - R_s)}{R_c(c + s)^2} \qquad (1)$$

or $$\frac{(R_c - R_s)}{R_c(r/q)}s = (c + s)^2 \qquad (2)$$

This is an expression for the reproduction path in terms of r/q.

Further characteristics of point '4' are found by differentiation, as follows:

Represent the term $\frac{(R_c - R_s)}{R_c\,(r/q)}$ by 'z'.

Expression (2) then becomes $zs = (c + s)^2$

$c = \sqrt{z}\,\sqrt{s} - s$

$dc/ds = \frac{1}{2}\sqrt{z}\,s^{-1/2} - 1$ which equals zero at point '4', the maximum on the reproduction path.

$\frac{1}{2}\sqrt{z}\,s^{-1/2} - 1 = 0$

$s = \frac{1}{4}z$

$c = \sqrt{z}\,\sqrt{s} - s = \sqrt{z}\,\sqrt{z}/2 - \frac{1}{4}z = \frac{1}{4}z$

'c' and 's' are therefore always equal at the point '4'.

If F_4 represents total food consumed at the point '4' in unit time, then at point '4' $c = s = F_4/2$.

Solving for r/q in Exp. (1),

$$r/q = \frac{(F_4/2)(R_c - R_s)}{R_c\,(F_4/2 + F_4/2)^2} = \frac{R_c - R_s}{2R_c\,F_4}$$

From Exp. (2),

$$s = \frac{(R_c - R_s)}{2\,R_c\,F_4}\,\frac{R_c}{(R_c - R_s)}\,(c + s)^2 \text{ or } s = \frac{(c + s)^2}{2F_4} \qquad (3)$$

This is the expression for the ideal reproduction path in terms of F_4. Similarly, the expression for the ideal expansion path becomes

$$c = \frac{(c + s)^2}{2F_4} \qquad (4)$$

Since there is no body mass to be maintained at the very beginning of life, the ration at this point is composed entirely of creative component. At birth, our present starting point, the ration has varied so as to contain a certain portion of the sustaining component to maintain the body mass already developed. On the CS diagram, therefore, the tangent to the growth line at the origin has an angular displacement from the C-axis of $(R_c - R_y)$ where R_y represents the protein content of the first ration after birth (corresponding to egg yolk in the case of birds). Because of this displacement which continues between the reproduction and growth lines, as shown on Fig. 3, VA = BE.

UV is the line of equal R-line distribution.

Let 'c' and 's' be the creative and sustaining components at the point 'B'.

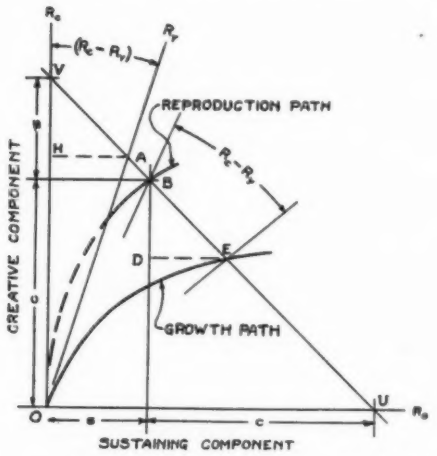

Fig. 3. Derivation of growth path from reproduction path.

$(c + s)$ at 'B' equals $(c + s)$ at 'E'

s at 'B' equals $\frac{1}{2F_4}(c + s)^2$ (Exp. 3)

s at 'E' equals $\frac{1}{2F_4}(c + s)^2 + DE$

$$DE = HA = \frac{(R_e - R_y)}{(R_e - R_s)}(OU) = \frac{(R_e - R_y)}{(R_e - R_s)}(c + s)$$

Therefore for growth line,

$$s = \frac{1}{2F_4}(c + s)^2 + \frac{(R_e - R_y)}{(R_e - R_s)}(c + s) \quad (5)$$

The displacement between the expansion line and the recession line is also angular to the same extent, so that for the recession line,

$$c = \frac{1}{2F_4}(c + s)^2 + \frac{(R_e - R_y)}{(R_e - R_s)}(c + s) \quad (6)$$

On Fig. 4, the path 3-4-5-2 represents the divergence from growth to accommodate reproduction. Without this divergence, the growth line, as approximated by males, would continue mathematically to its intersection with the sustaining component axis at '6'. At this point, the creative component of the ration becomes zero so that '6' represents the point of pure maintenance for living machines planned for growth without divergence of reproduction. When the females have passed along 3-4-5-2, they have reached a pivot between reproduction and further growth. Both activities have a momentary value of zero so that this is also a point of pure maintenance following reproduction.

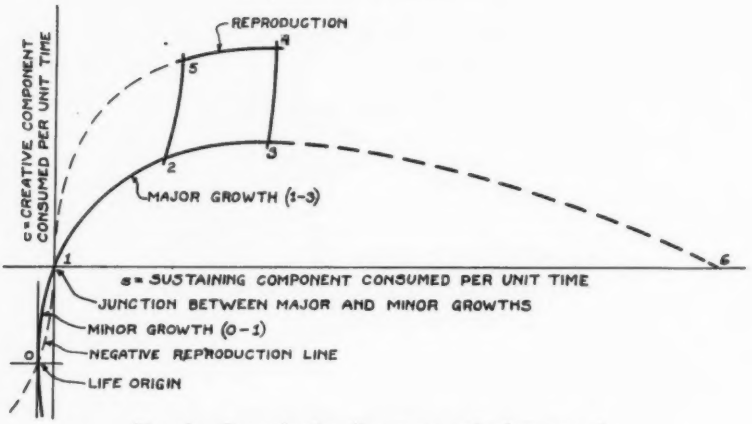

Fig. 4. Reproductive divergence and minor growth.

The locations of points '2', '3', '4', '5' and '6' are found by solving for the intersections of the various lines. For point '2', add expressions (5) and (6):

$$(c+s)_2 = F_4\left(1 - \frac{2(R_c - R_y)}{R_c - R_s}\right) \tag{7}$$

For point '3', add expressions (5) and (4):

$$(c+s)_3 = F_4\left(1 - \frac{R_c - R_y}{R_c - R_s}\right) \tag{8}$$

For point '4', add expressions (4) and (3):

$$(c+s)_4 = F_4 \tag{9}$$

For point '5', add expressions (3) and (6):

$$(c+s)_5 = F_4\left(1 - \frac{R_c - R_y}{R_c - R_s}\right) \tag{10}$$

For point '6', solve growth expression when $c = 0$:

$$(c+s)_6 = s_6 = 2F_4\left(1 - \frac{R_c - R_y}{R_c - R_s}\right) \tag{11}$$

The value of $(c + s)$ at point '1' is zero.

Note that the $(c + s)$ margin from '3' to '6' is the same as that from '1' to '3'. This agrees with the notion that the distance from maximum growth activity to zero growth activity should be the same as the distance from zero growth activity to maximum growth activity. It suggests that change in $(c + s)$ has something to do with season and time and leads to the conclusion that the $(c + s)$ distance from '3' thru '4' and '5' to '2' should also be equal to the $(c + s)$ distance from '3' to '6'—both comprising the movement from peak growth to point of mainten-

ance. Expressing this in mathematical form,

$(c+s)_3=(c+s)_4-(c+s)_3+(c+s)_4-(c+s)_2$

or $2(c+s)_3=2(c+s)_4-(c+s)_2$ (12)

Substituting for $(c+s)$ in Exp. (12) the values given in expressions (7) to (11) inclusive,

$$2F_4\left(1-\frac{R_c-R_y}{R_c-R_s}\right)=2F_4-F_4\left(1-\frac{2(R_c-R_y)}{R_c-R_s}\right)$$

which clears to $$\frac{R_c-R_y}{R_c-R_s}=\frac{1}{4} \qquad (13)$$

Applying expression (13) to expressions (7) to (11) inc.

$(c+s)_2=F_4\,(1-2/4)=\frac{1}{2}F_4$
$(c+s)_3=F_4\,(1-1/4)=\frac{3}{4}F_4$
$(c+s)_4=F_4$
$(c+s)_5=F_4\,(1-1/4)=\frac{3}{4}F_4$
$(c+s)_6=2F_4\,(1-1/4)=1\frac{1}{2}F_4$

It is noted that these values have a common measure of $\frac{1}{4}F_4$. In order to clear of fractions, designate this measure as 'f' so that $f=\frac{1}{4}F_4$. ('f' will also be shown later as the amount of change in $(c+s)$ during minor growth.) The values of $(c+s)$ at the points '2' to '6' then become,

$(c+s)_2=2f$
$(c+s)_3=3f$
$(c+s)_4=4f$
$(c+s)_5=3f$
$(c+s)_6=6f$

By applying $F_4=4f$ and $\frac{R_c-R_y}{R_c-R_s}=\frac{1}{4}$ to expressions (5), (4), (3) and (6), the expressions for the paths of the cycle become,

For growth, $$s=\frac{1}{8f}(c+s)^2+\tfrac{1}{4}(c+s) \qquad (14)$$

For expansion, $$c=\frac{1}{8f}(c+s)^2 \qquad (15)$$

For reproduction, $$s=\frac{1}{8f}(c+s)^2 \qquad (16)$$

For recession, $$c=\frac{1}{8f}(c+s)^2+\tfrac{1}{4}(c+s) \qquad (17)$$

MINOR GROWTH

The activities so far treated correspond approximately to post-natal life. Time of birth itself has shifted to suit environment and the intelligence and care of the progenitor so that the actual time of emergence has little significance. Now consider what might be called "minor growth"—corresponding approximately to prenatal growth.

To this end, continue the growth line into the third quadrant as is done in Fig. 4 until the element of the line is parallel to the C-axis. This is the true origin of the representation as far as complete life is concerned. At this point, 'O', all activity is creative and the body mass is zero. In other words, this is the mathematical representation of the beginning of life. Solving for the position where the element of the growth line is parallel to the C-axis gives $(c + s) = -f$. The beginning of life should also lie on the negative (receiving) reproduction line and solving for the position of intersection between $s = \frac{1}{8f}(c + s)^2 + \frac{1}{4}(c + s)$ and $s = -\frac{1}{8f}(c + s)^2$ also gives $(c + s) = -f$.

Between point '0' and point '1', there is a gain in $(c+s)$ of $0 - (-f)$ or f. This represents the vital power acquired by progeny from progenitor. According to Third Criterion, this gain should be equal to the vital power lost by the progenitor during reproduction. Such loss is found between the points '3' and '2'. At point '3', the measure of vital power is 3f. The living machine then moves thru points '4' and '5' to point '2' where the measure of vital power is 2f. The loss of vital power during reproduction is therefore $3f - 2f$ or f. Thus vital power lost during reproduction is found to be exactly equal to that received during minor (prenatal) growth. This satisfies Third Criterion.

WEIGHT-AGE EXPRESSION FOR MAJOR GROWTH

It was noted in the development of expression (12) that change in $(c + s)$ has something to do with elapsed time. 'c' and 's' represent food consumed per unit time and their expressions have been shown to be parabolic. It seems logical therefore to assume that the relationship between $(c + s)$ and time is also parabolic. $(c + s)$ increases with size but size is opposed by the force of gravity. This indicates that the axis of the assumed parabola is normal to the time axis and that the apex of the parabola is uppermost. Such a parabola is shown on Fig. 5.

Reproductive Divergence, 'd', (page 7) is taken to be the portion of 'peak food capacity for sexless growth' which is diverted for reproduction. It has a value, in ideal reproduction, of

$$d = \frac{F_6 - F_4}{F_6} = \frac{6f - 4f}{6f} = \frac{1}{3} \qquad (18)$$

In other words, since the peak food capacity for sexless growth is 6f and that for the female is 4f, the expression $6(1-d)f$ satisfies both conditions and becomes the height of the apex of the parabola on Fig. 5. The focal distance is represented by af with the value of 'a' not known.

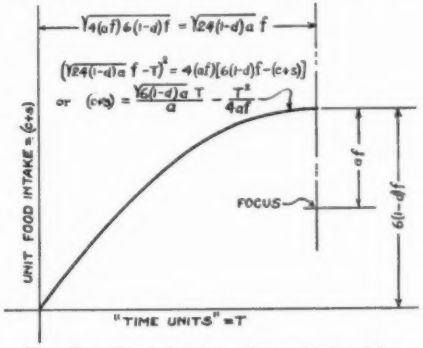

Fig. 5. Food intake—time relationship.

Let 'T' be the 'time units' along the time axis with a scale as derived from the parabola and 'A' the age in terms of one of our arbitrary time divisions. Let 'h' be the age at which mature weight is reached—the same divisions of time being used for both 'A' and 'h'. As shown on Fig. 5, the number of 'time units' required to reach mature weight is $\sqrt{24(1-d)a}\ f$. Therefore the conversion expression between 'T' and 'A' becomes

$$T = \frac{\sqrt{24(1-d)a}\ f\ A}{h} \tag{19}$$

$$(c+s) = \frac{\sqrt{6(1-d)a}\ T}{a} - \frac{T^2}{4af} \quad \text{(from Fig. 5)} \tag{20}$$

Substituting (19) in (20)

$$(c+s) = \frac{6(1-d)f}{h}\left(2A - \frac{A^2}{h}\right) \tag{21}$$

Structural materials being delivered to a building cite are a measure of the size of apartment building being built. Similarly the weight of creative component consumed by an efficient living machine during growth is a measure of the weight of the living machine being grown.

The growth expression (14) was found to be

$$s = \frac{1}{8f}(c+s)^2 + \tfrac{1}{4}(c+s) \qquad \text{or}$$

$$c = (c+s) - s = 3/4\ (c+s) - 1/8f\ (c+s)^2 \tag{22}$$

This is a measure of allotment for growth. It is a function of the

differential of body weight with regard to the differential of food intake. If 'W_h' represents body weight at end of growing period and 'W' the weight at any point,

$$\frac{dW}{d(c+s)} = \frac{W_h}{X}\left[3/4\ (c+s) - \frac{(c+s)^2}{8f}\right]$$

$$W = \frac{W_h}{X}\left[3/8\ (c+s)^2 - \frac{(c+s)^3}{24f}\right] \qquad (23)$$

$W = W_h$ when $(c+s) = 6(1-d)f$

Applying these values to Exp. (23),

$$X = \frac{9}{2}f^2\ (1-d)^2\ [3-2(1-d)] \qquad (24)$$

Substituting (21) and (24) in (23) and clearing,

$$W = \frac{W_h\, 2(1-d)}{[3-2(1-d)]h^3}\left[\frac{3h}{2(1-d)}\left(2A - \frac{A^2}{h}\right)^2 - \left(2A - \frac{A^2}{h}\right)^3\right] \qquad (25)$$

This expression, developed according to conditions assumed to be those of highest efficiency, is therefore the expression for most efficient growth during the major growth period. It determines weight in terms of the one natural variable, age. "h" and "W_h" are inherently constant according to family and strain. "d" is constant according to sex.

VARIATION IN TYPE OF LIVING MACHINE

The living machine considered, so far, has been one capable of growth and reproduction with the reproductive activity ending at birth of offspring. This is the bird-type machine. The mammal-type machine functions in growth and reproduction but the reproductive activity continues for some time after birth in the form of lactation. The purpose of this is to add to the vital power bestowed on offspring up to the time of birth. The effect mathematically is to elevate all reference points of the cycle. This lengthens all paths. It increases ultimate body mass, makes possible a greater degree of reproduction to the succeeding generation and increases the debt to the preceding generation by the same degree. The characteristics of the cycle apparently remain the same as for the bird-type machine.

So, if the living machines developed in the "trial and error" laboratory of Nature are highly efficient, as they seem to be, and if the interpretations, assumptions and mathematical steps followed in this paper have been properly directed, the weight-age expression (Exp. 25) should co-ordinate closely with observed data. There should be recorded evidence of minor as well as major growth. Also, since the expression contains the factor of reproductive divergence, 'd', it should apply to female and male alike, with 'd' valued at 1/3 (Exp. 18) for the female and at zero (approx.) for the male.

Birth, to which age is generally referred, does not coincide with the start of major growth and the zero of weight is located at the start of minor growth. Adjustment for this variance may be made either by direct shift of axes or by deducting from observed weight and age readings the weight and age at start of major growth. Since Exp. (25) has been developed entirely from abstract considerations, it is not tied to any arbitrary system of units. It is only necessary that W and W_h be expressed in the same unit of weight and A and h in the same unit of time.

COMPARISON WITH OBSERVED DATA

On applying the expression for major growth to the weight data on chickens, it is found that start of major growth appears located neither at start of incubation nor at time of hatching but about one-half week before hatching. This agrees with findings previously made by others:

1. Bohr and Hasselbalch, Lawson and Edwards, and Atwood and Weakley (W. Va. Ag. Exp. Sta. Bul. 185) "determined that the increase of carbon dioxide during the period of incubation was proportionate to the increase in the weight of the developing embryo" and that the line representing the release of carbon dioxide compared to age was *sigmoid*.

2. At about the 18th day of incubation, the nutrient for the unhatched chick shifts from albumen to yolk and the yolk sac enters the body. (Poultry Production, Lippincott and Card.)

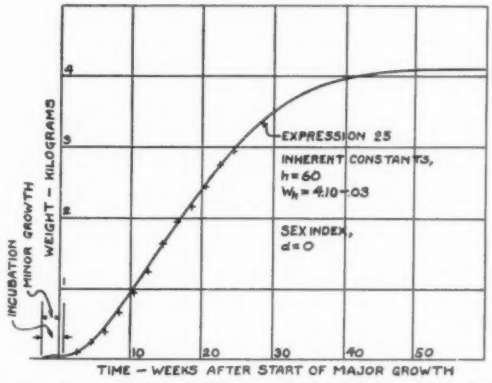

Fig. 6. Major growth expression (25) compared with data on hybrid cockerels by Jull and Titus. (Journal of Agriculture Research, **36**: 6)

3. A decline in growth rate has been observed between the 15th and 18th days. (Poultry Production, Lippincott and Card.)

These observations agree with our mathematical finding that two separate phases of growth exist (both sigmoid). Fig. 6 shows the weight expression for males (d = 0) applied to hybrid cockerels as

found by Jull and Titus. Fig. 7 shows the weight expression for females ($d = 1/3$) applied to Leghorn pullets as found by Hurd. It is noted that two weight systems are used in these cases without the

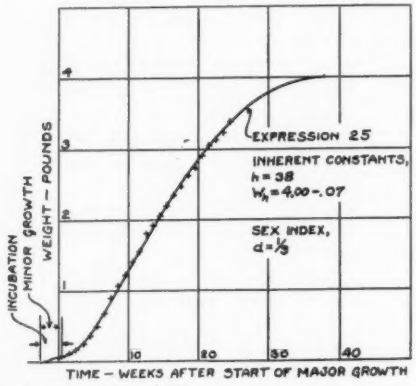

Fig. 7. Major growth expression (25) compared with data on Leghorn pullets by L. M. Hurd (Practical Poultry Farming).

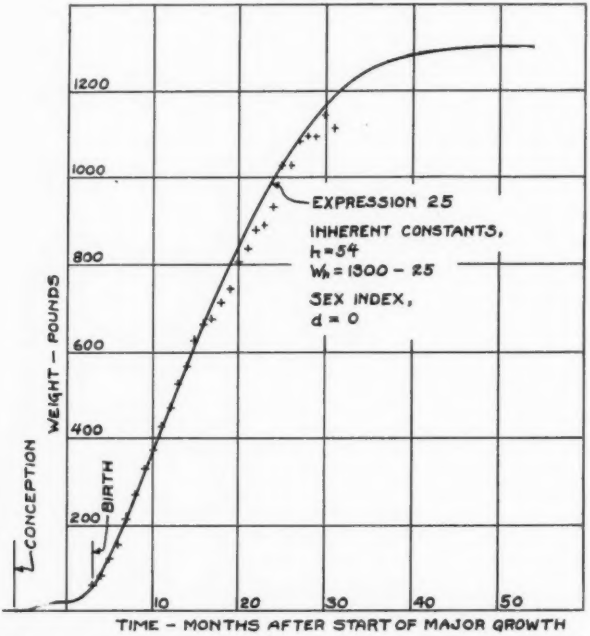

Fig. 8. Major growth expression (25) compared with data on Jersey bull by C. H. Eckles and Associates. (Mo. Agr. Exp. Sta. Res. Bul. 96).

need of empirical exponents or coefficients and that the corroboration is close.

Fig. 8 shows application to weight data on a Jersey bull taken by Eckles and Associates. The shift from minor growth to major growth is here shown to take place at 3 months before birth. This agrees with the finding of Ragsdale, Elting and Brody (Mo. Agr. Exp. Sta. Res. Bul. 96) who report a definite change in weight trend of cows at about the 6th month of gestation.

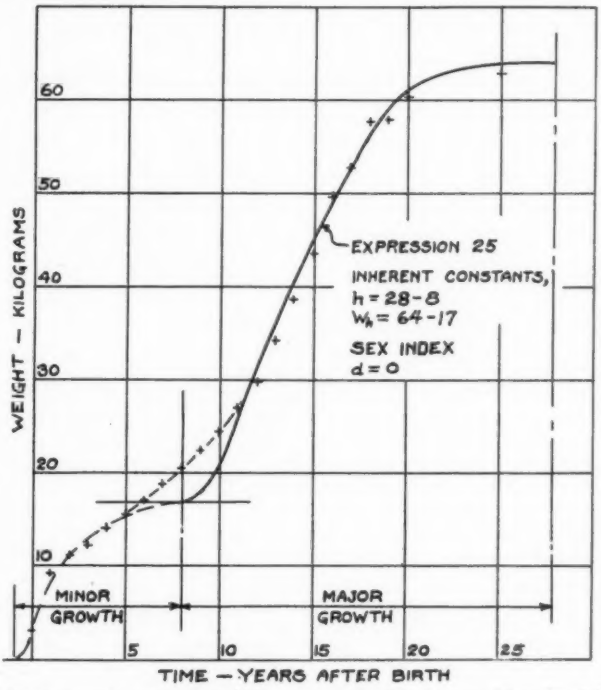

Fig. 9. Major growth expression (25) compared with data on Man (male) by Quetelet (Growth and Form, D. W. Thompson).

Fig. 9 shows comparison with weight data on Man (male) taken by Quetelet. It is noted that the change from minor to major growth occurs very late—at about eight years of age. Full depth of the theoretical "valley" is not reached between the ages of 5 and 11 years but the junction brings about the undulated appearance characteristic of the weight-age curve for Man. It appears as something more than coincidence that the deciduous or "baby" teeth are shed between the ages of 6 and 11 years, during the shift from minor to major growth.

CONCLUSION

A limited investigation is made of a perfect elementary living machine. Growth and reproduction under the assumed conditions are shown to be paths of a four path cycle. Mathematical expressions for the paths are developed depicting the continuous exchange of vital power from generation to generation.

The expression of the path of growth is integrated to produce the expression of most efficient growth. When compared with observed data, this expression is found to describe actual growth with remarkable closeness. The single expression traces both the increasing and decreasing segments of growth in true proportion. This indicates that life in becoming more and more efficient has also approached closer and closer to the mathematical ideal of the perfect elementary living machine.

It would seem then that attention to the elementary growth-reproduction cycle should prove of value in research into living performance.

THE EFFECTS OF PROLONGED EXPOSURE TO LOW TEMPERATURE ON THE BODY GROWTH AND ON THE WEIGHTS OF ORGANS IN THE ALBINO RAT

By

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Four years ago we undertook to study the effect of environmental temperature on the rat. The data on the basal metabolism have been published (Schwabe et. al., 1938). In this paper we present the data pertaining to body weights, body measurements, organ weights, and estrous cycles.

PROCEDURE AND METHODS

The rats used in these experiments (of Wistar Stock inbred for many generations) were normal albino females and males of approximately sixty-five days of age at the beginning of the experiment. As may be seen from Table I, the major part of the work was done with three groups of fifty female rats over periods of fifteen, thirty, and sixty days. Each group of fifty was subdivided into a cold series of twenty-five animals exposed to cold and a warm series of twenty-five littermates regarded as controls. This made a total of seventy-five controls and seventy-five animals exposed to cold. In addition, for the purpose of comparing sexes, we included a 60-day group of male rats similarly divided and chosen from the same litters as the 60-day females (Table III).

The controls or warm series lived at all times in a room having a constant average temperature of 26°C. with a variation of ±2°C. The rats exposed to cold or cold series were kept in a room having a constant temperature of 1.6°C. with a variation of ±1°C. for sixteen hours from 4 P. M. to 8 A. M., and removed to the constant temperature warm room for eight hours, from 8 A. M. to 4 P. M. The average low temperature for the twenty-four hours for all animals exposed to the cold was 9.7°C. Food and water, and the time exposed to light were the same in both the control, warm series and the experimental, cold series. A more detailed account of the procedure used in caring for the rats was previously published (Schwabe et. al., 1938). Let us again point out that the only difference in the treatment of the two series was one of temperature.

At the end of the experiment the animals were autopsied immediately

TABLE I

ACTUAL WEIGHTS OF BODY AND CARCASS IN GRAMS AND OTHER WEIGHTS IN MILLIGRAMS OF TWENTY-FIVE CONTROL RATS (WARM SERIES) AND OF TWENTY-FIVE RATS EXPOSED TO COLD FOR FIFTEEN, THIRTY AND SIXTY DAYS. ALL MEASUREMENTS IN MILLIMETERS.

ORGAN	DAYS DURATION OF EXPERIMENT	MEAN WEIGHTS		Per Cent Change	Mean Diff. / S. E. of Diff.	RANGE	
		Warm Series	Cold Series			Warm Series	Cold Series
Body	15	131.3	131.0	— —	— —	105-152	108-150
	30	144.8	138.8	− 4.1	2.1	122-165	124-156
	60	168.0	152.0	−10.7	2.4	140-197	130-194
Carcass	15	105.0	101.2	− 3.5	1.4	81-120	94-118
	30	117.6	109.2	− 7.1	3.2	101-139	93-124
	60	133.4	114.3	−16.7	4.4	111-159	102-148
Nose to Anus	15	172.0	171.2	— —	— —	163-181	160-179
	30	178.4	172.4	− 3.4	3.9	167-190	163-183
	60	*184.5	180.3	− 2.3	2.3	175-198	168-188
Anus to Tail Tip	15	151.9	150.4	— —	— —	140-160	136-155
	30	154.8	152.4	− 1.6	1.0	141-168	140-172
	60	157.6	153.4	− 2.7	2.1	142-173	140-164
Nose to Tail Tip	15	324.0	314.8	— —	— —	309-335	302-334
	30	332.8	326.4	− 1.9	3.1	315-342	308-337
	60	342.0	333.0	− 2.9	2.8	314-366	320-341
Diameter of Tail	15	7.4	7.4	— —	— —	7.0-8.0	6.7-8.0
	30	7.9	7.7	− 2.8	1.7	7.2-8.8	7.0-8.3
	60	8.0	7.8	− 2.8	0.2	7.0-9.0	7.0-10.0
Kidney	15	1093.6	1192.0	+ 9.0	2.9	978-1280	1031-1395
	30	1184.8	1258.0	+ 6.2	2.1	964-1500	1099-1358
	60	1361.2	1493.6	+ 9.6	2.4	1065-1815	1295-1730
Spleen	15	888.4	885.6	— —	— —	445-1262	401-1655
	30	939.2	758.8	−19.2	2.3	514-1720	436-1158
	60	761.6	723.0	− 5.1	0.5	385-1460	345-1195
Ovary	15	45.4	44.3	− 2.0	0.3	25-58	23-75
	30	51.0	43.5	−14.7	2.3	24-67	22-77
	60	54.0	47.1	−12.8	1.8	28-101	27-63
Uterus	15	308.8	244.8	−20.7	2.1	147-665	136-448
	30	342.4	223.6	−34.7	3.3	165-855	115-709
	60	359.0	268.0	−25.2	1.6	140-1010	70-1180
Pituitary	15	6.8	6.6	− 3.0	0.6	4.5-9.0	4.5-8.0
	30	7.8	7.0	−10.3	2.1	5.5-11.0	5.5-9.5
	60	9.7	8.9	− 8.0	1.4	7.5-17.0	6.0-11.5
Thyroid	15	10.9	12.4	+13.2	3.5	8.5-13.5	9.0-15.0
	30	11.0	12.4	+10.9	2.6	8.5-13.5	6.5-17.0
	60	11.1	13.2	+18.2	3.6	9.0-15.0	10.0-19.5
Adrenals	15	45.6	52.1	+14.1	3.5	33-64	36-76
	30	48.4	50.1	+ 3.5	0.7	30-73	33-69
	60	50.3	54.0	+ 7.9	1.8	32-68	37-75
Brain	15	1679.4	1672.8	— —	— —	1401-1800	1540-1832
	30	1711.2	1685.2	− 1.6	1.1	1555-1873	1456-1800
	60	1734.0	1678.0	− 3.1	2.7	1555-1925	1515-1795
Heart	15	590.8	675.6	+14.3	4.4	492-729	565-788
	30	634.0	702.4	+10.8	4.2	541-780	580-867
	60	658.0	698.0	+ 6.5	1.6	510-840	550-1045
Lungs	15	950.0	1074.0	+13.1	3.0	784-1161	933-1753
	30	1084.6	1098.0	+ 1.4	0.5	908-1610	893-1265
	60	1080.0	1106.0	+ 2.5	0.8	860-1340	925-1505
Thymus	15	275.8	286.0	+ 4.0	0.5	171-507	144-407
	30	330.0	302.0	− 8.5	1.3	122-510	179-486
	60	329.0	284.0	−13.6	2.3	165-520	190-400
Intestinal Tract	15	10.6	12.6	+18.8	4.2	8.0-13.5	9.8-17.3
	30	11.7	13.2	+13.0	3.0	8.6-16.7	9.9-19.4
	60	15.8	19.7	+25.6	3.3	7.4-24.3	8.3-28.1
Liver	15	5075.2	5581.2	+10.0	3.3	4200-6110	4618-7015
	30	5273.2	5508.0	+ 4.4	1.3	4148-7146	4756-6938
	60	7017.6	6820.0	− 2.8	0.6	4845-10814	5385-9095

TABLE II

BODY AND CARCASS WEIGHTS IN GRAMS; MEASUREMENTS IN MILLIMETERS; AND ORGAN WEIGHTS IN MILLIGRAMS. THE FIRST LINE OF FIGURES OPPOSITE EACH ORGAN IS BASED ON ACTUAL WEIGHTS; UNDER THIS, ORGAN WEIGHTS PER 100 GRAMS BODY WEIGHT. SEVENTY-FIVE FEMALE RATS IN EACH SERIES. COEFFICIENT OF VARIATION $= \frac{\sigma \times 100}{\text{MEAN}}$. STANDARD ERROR OF DIFFERENCE $= \sqrt{(\text{S.E.}^1)^2 + (\text{S. E.}^2)^2}$. S. E. = STANDARD ERROR OF MEAN $= \frac{\text{S.D.}}{\sqrt{N}}$

ORGAN	MEAN		Per Cent Change	Mean Diff.	Coefficient of Variation	
	Warm Series	Cold Series		S. E. of Diff.	Warm Series	Cold Series
Body	152.1	145.9	− 4.1	2.1		
Carcass	120.3	111.5	− 9.3	4.3		
Nose to Anus	178.0	174.7	− 1.8	3.1		
Anus to Tail Tip	154.7	152.0	− 0.8	1.7		
Nose to Tail Tip	330.6	326.0	− 1.2	2.6		
Diameter Tail Root	7.7	7.6	− 0.9	1.0		
Kidneys	1215.3 799.2	1314.6 901.0	+ 8.2 +12.7	4.5 6.7	11.1	10.9
Spleen	862.0 566.7	786.0 538.7	− 8.8 − 4.9	3.2 1.6	19.3	19.1
Ovaries	50.3 33.1	44.8 30.7	−11.0 − 7.0	5.7 3.6	12.2	12.6
Uterus	330.0 217.0	244.0 167.5	−26.1 −18.1	7.8 6.7	21.4	26.7
Pituitary	8.1 5.3	7.5 4.1	− 8.2 − 4.3	3.6 1.8	13.4	16.2
Thyroid	10.9 7.1	12.5 8.6	+15.0 +20.3	9.4 12.2	7.4	10.4
Adrenals	48.1 31.6	52.1 35.7	+ 8.3 +12.9	5.3 8.2	8.5	9.6
Brain	1706.7 1123.0	1680.0 1152.0	− 1.5 + 2.6	3.9 6.4	2.3	2.6
Heart	623.0 409.6	694.2 475.8	+11.4 +16.2	11.5 15.9	5.3	6.1
Lungs	1033.4 679.5	1095.4 750.8	+ 6.0 +10.5	5.0 8.5	7.8	6.7
Thymus	314.0 206.4	290.6 199.2	− 7.4 − 3.5	3.1 1.4	16.8	13.7
Intestinal Tract	12.8 8.4	15.0 10.3	+17.3 +22.2	3.3 6.0	20.6	20.0
Liver	5758.0 378.6	5940.7 407.2	+ 3.1 + 7.6	1.3 3.1	17.0	11.2

TABLE III

BODY AND CARCASS WEIGHTS IN GRAMS OF CONTROL RATS (WARM SERIES) AND OF RATS EXPOSED TO COLD FOR SIXTY DAYS; MEASUREMENTS IN MILLIMETERS; AND ORGAN WEIGHTS IN MILLIGRAMS. THE FIRST LINE OF FIGURES OPPOSITE EACH ORGAN IS BASED ON ACTUAL WEIGHTS; UNDER THIS, ORGAN WEIGHTS PER 100 GRAMS BODY WEIGHT. TWENTY-FIVE MALE RATS IN EACH SERIES. COEFFICIENT OF VARIATION $= \frac{\sigma \times 100}{\text{MEAN}}$.

STANDARD ERROR OF DIFFERENCE $= \sqrt{(\text{S.E.}_1)^2 + (\text{S. E.}_2)^2}$. S. E. = STANDARD ERROR OF MEAN $= \frac{\text{S.D.}}{\sqrt{N}}$.

ORGAN	MEAN		Per Cent Change	Mean Diff. / S. E. of Diff.	RANGE		Coefficient of Variation	
	Warm Series	Cold Series			Warm Series	Cold Series	Warm Series	Cold Series
Body	234.4	214.4	− 8.5	2.2	162-288	164-299	14.5	14.0
Carcass	194.2	173.2	−10.9	2.6	127-236	132-252	15.3	16.1
Nose to Anus	206.4	202.4	− 1.9	1.4	187-224	184-226	5.5	4.7
Anus to Tail Tip	168.0	158.8	− 5.5	4.4	149-170	148-184	4.9	4.1
Nose to Tail Tip	274.4	361.2	− 3.5	3.1	341-389	351-407	4.1	3.9
Diameter Tail Root	9.3	8.9	− 4.2	2.5	7.9-10.0	8.0-10.0	7.0	6.2
Kidneys	1704.0 727.0	1846.8 861.4	+ 8.4 +18.5	1.8 3.8	1481-2573	1018-2365	17.7	14.4
Spleen	916.8 391.1	892.0 416.1	− 2.7 + 3.6	.40 .50	520-1417	629-1410	22.4	27.8
Testes	2688.0 1147.0	2592.8 1209.0	− 3.6 + 5.4	1.1 1.5	2200-3277	2180-3290	11.9	12.8
Epididymides	904.0 385.7	827.6 386.0	− 8.6	2.5	696-1056	654-1087	11.6	13.5
Pituitary	7.4 3.2	7.2 3.4	− 2.6 + 6.4	0.6 1.4	5.5-9.0	5.5-9.5	18.3	11.2
Thyroid	12.9 5.5	15.9 7.4	+23.2 +34.8	3.6 5.2	11.0-22.5	10.5-20.0	19.9	20.0
Adrenals	37.0 15.8	42.6 19.9	+15.1 +26.0	3.3 5.5	30-58	31-45	15.7	14.2
Brain	1803.6 76.9	1794.0 84.7	− 0.5 + 8.8	0.3 5.7	1661-2030	1667-2004	4.7	5.6
Heart	892.0 380.5	929.6 433.7	+ 4.2 +14.0	1.1 3.5	732-1217	698-1300	12.8	13.9
Lungs	1363.6 582.2	1436.8 670.3	+ 5.4 +15.1	1.3 3.6	1111-1720	1058-1827	15.2	12.2
Thymus	316.8 132.1	315.2 147.0	+11.3	1.3	175-559	233-415	27.4	30.7
Intestinal Tract	15.2 6.5	16.0 7.5	+ 5.2 +14.8	1.2 3.2	10.7-21.0	9.8-19.7	15.0	15.3
Liver	7068.0 301.5	7265.2 338.9	+ 2.8 +12.4	0.7 3.2	6425-10235	5097-9083	14.6	11.4

after their basal metabolic rate had been determined and after fasting at least twenty-five hours (Schwabe et. al., 1938). After ether anesthesia, body measurements were taken, the heart was exposed and the blood aspirated therefrom as completely as possible by means of a hypodermic syringe. The organs were then carefully dissected free of adherent tissue, placed in closed vials and weighed immediately. The organs were always prepared for weighing by the same person; this gave the most uniform procedure we could devise.

RESULTS

Body Weights: The body weight obtained at the time the basal metabolic rate was determined has previously been referred to and the amount of food eaten has been discussed (Schwabe et. al., 1938). The weights here reported were obtained at autopsy and within an hour after the rats were removed from the metabolism chamber. Although the growth curve of the animals exposed to cold was slightly below that of the controls, the mean body weights at autopsy were not significantly different. The mean difference divided by the standard error of the difference (subsequently referred to as the "significant ratio") was obtained; and if this figure was three or more, the means were considered to be significantly different. The figures thus obtained for body weights were 2.1, 2.4, and 2.2 in the 30-day female, 60-day female, and male groups respectively (Tables I and III); and 2.1 when the seventy-five females were combined into one group (Table II). The weights in the 15-day group were nearly identical in both series. This should not be interpreted as indicating that no effect was produced by this length (fifteen days) of exposure to cold; on the contrary, the organ weights in this group were in many cases significantly altered in weight.

Carcass: The carcass represents the body remaining after all the organs and blood had been removed. The total weight of viscera thus removed was greater in the rats exposed to cold. This is evident by the per cent difference in carcass weight, which amounted to 3.5 per cent in the 15-day females, 7.1 per cent in the 30-day females, 16.7 per cent in the 60-day females, 9.3 per cent in the combined group of seventy-five females, and 10.9 per cent in the male group (Tables I, II, and III). Also, in contrast to the body weight, which was not significantly different in any group exposed to cold, the mean carcass weight was significantly different in the 30- and 60-day female groups (Table I) and in the combined female group (Table II), the "significant ratio" being 3.2, 4.4, and 4.3 respectively in the three cases.

Body Measurements: The measurements of body lengths, tail lengths, nose to anus lengths, and nose to tail-tip lengths were made

with the rats under deep anesthesia. Care was taken to get the animal flat on the paper during the measurements; this precaution Donaldson (1924) emphasized in his treatise on the rat. The tail root at the hair line was measured with calipers. The data from these four measurements need be discussed only briefly since the significant changes were evident in only three instances in the 30-day female group and twice in the male group. In the 30-day group of females, the first was the nose to anus length which gave means of 178.4 and 172.4 millimeters and a "significant ratio" of 3.9 (Table I). The second was obtained in the nose to tail-tip measurement where the means were 332.8 in the warm series and 326.4 millimeters in the cold series with a "significant ratio" of 3.1 (Table I). When the 15-, 30-, and 60-day groups were considered together, a significant change occurred again in the nose to anus measurement. Although the bodies of the rats exposed to cold were only 1.8 per cent shorter than their litter-mate controls, this change was significant, as shown by a "significant ratio" of 3.1 (Table II). In the males the tails of rats exposed to cold were 5.5 per cent shorter than the controls, the "significant ratio" being 4.4. This difference was also reflected in the nose to tail-tip measurements as shown by a mean percentage difference of 3.5 per cent and a "significant ratio" of 3.1 (Table III). These changes, although considered significant, are small and perhaps should not be further emphasized. Yet there seems to be unquestionable evidence that mice grown for a long time in a cold environment have shorter bodies and tails than the controls kept in a warm room (Sumner, 1909; Ogle, 1934).

Kidneys: In all three groups of female rats (Table I) and also in the males (Table III) the kidneys were heavier in the rats exposed to cold. The data, uncorrected for differences in body weight, between the warm series and cold series show that the kidneys were not significantly different in weight when the three female groups were considered separately (Table I), but became significantly different when the three female groups were considered together; as shown in Table II the "significant ratio" was 4.5. This significant difference was more striking when the weights of the kidneys were corrected to a hundred-gram body weight basis. The kidneys of the cold series were 12.7 per cent larger, with a "significant ratio" of 6.7 (Table II). A similar comparison in the male group showed the kidneys to be 8.4 per cent and 18.5 per cent larger, and a "significant ratio" of 1.8 and 3.8 for the uncorrected and corrected data respectively (Table III).

Spleen: We were much surprised to find nearly identical mean weights in an organ like the spleen which often varies greatly in size. Both the control rats and those exposed to cold showed nearly identical mean weights in the 15- and 60-day female groups. In the 30-day

group the spleens were 19.2 per cent smaller in the cold series, but the greater variability in weight prevented this difference from being significant, as shown by a "significant ratio" of 2.3 (Table I). When all female groups were combined the figures, uncorrected for differences in body weight, showed the spleen to be significantly smaller in the cold series; but this difference, which amounted to —8.8 per cent largely disappeared when the data were corrected to a hundred-gram body weight basis, the "significant ratio" being 3.2 and 1.6 respectively (Table II). The data for the male rats further confirmed the conclusion that the weights of the spleens were not materially altered by cold, the mean weights of the warm series and the cold series being nearly identical (Table III). A contraction of the spleen in the living animal, induced by sudden changes in environmental temperature, has been reported by Bancroft and Elliott (1936). Thus, it is conceivable that long exposure to cold may result in a slightly reduced weight of the spleen, a reduction which persists for a time even after the animal is returned to a warm room environment.

Ovaries: The ovaries were smaller in all three groups exposed to cold (Table I). The differences in weight between the ovaries of the cold series and the ovaries of the warm series were not significant, yet the percentage difference was high, being 2.0, 14.7, and 12.8 per cent in the 15-, 30-, and 60-day groups respectively (Table I). These figures were larger than those obtained after correcting for differences in body weights. When the three groups were combined, as in Table II, the ovaries were significantly smaller in the rats exposed to cold, the "significant ratio" being 5.7; and even after corrections were made for differences in body weight it was still significant (3.6, Table II).

Uterus: The mean weights of the uteri were 20.7, 34.7, and 25.2 per cent smaller in the three groups exposed to cold for fifteen, thirty, and sixty days respectively (Table I). The weights were so variable that significant changes were evident only in the 30-day group which showed a "significant ratio" of 3.3 (Table I); but when the three groups were combined, the "significant ratio" was 7.8 for the uncorrected data and 6.7 after corrections were made for differences in body weight(Table II).

Estrous Cycles: As one might infer from the weights of the ovaries and uterus the rats exposed to cold showed definite inhibition of estrus. These rats were sixty-five days of age and therefore were sexually mature at the time the experiment was started. The mean per cent of time in estrus was obtained by the method previously described (Emery, 1931) and was found to be definitely lower in the rats exposed to cold, the figures being 35.6 and 41.0 per cent respectively, with a "significant ratio" of 4.5 (Table IV). Furthermore, the mean length of the estrous

TABLE IV

THE ESTROUS CYCLES OF SEVENTY-FIVE FEMALE RATS EXPOSED TO COLD AND SEVENTY-FIVE CONTROLS ARE SHOWN WITH THE PER CENT OF TIME IN ESTRUS AND THE LENGTH OF THE ESTROUS CYCLE IN DAYS.

Types of Donors	Per Cent of time in Estrus				Length of Estrous Cycles			
	Range	Median	Mean	Mean Diff. / S. E. of Diff.	Range	Median	Mean	Mean Diff. / S. E. of Diff.
Warm Series	20 to 56	40.0	41.0	4.5	2 to 15	4.8	5.2	4.0
Cold Series	20 to 48	36.0	35.6		2 to 28	5.3	6.1	

cycle was longer in the rats exposed to cold, the means being 6.1 and 5.2 days, and the "significant ratio" 4.0. It is interesting to note in this connection that Ogle (1934) found no change in the age of sexual maturity of mice, as judged by the opening of the vaginal canal, when reared at a temperature of 64°F. Likewise, it is interesting to consider our findings in light of those of Sundstroem (1927) who, in his excellent review on reproduction in tropical climates, gives evidence of early sexual maturity in human beings living in warm climates.

Testes and Epididymides: These organs were not significantly changed in weight by exposing the rats to cold. The testes of the cold series were 3.6 per cent smaller before and 5.4 per cent larger after corrections were made for differences in body weight (Table III). The epididymides were 8.6 per cent smaller in the cold series, which is approximately the difference of 8.5 per cent observed in body weight. Therefore, per hundred grams body weight, the weights are the same in the warm and cold series, being 385.7 and 386.0 milligrams respectively (Table III).

Pituitary Gland: The pituitary was not significantly changed in weight in any of the groups. As shown in Table I, the pituitary glands of female rats exposed to cold for fifteen, thirty, and sixty days were somewhat smaller than those of the controls. When these three groups were combined (Table II), the figures uncorrected for body weight showed the mean pituitary weight to be significantly smaller in the controls. But when corrections were made for differences in body weight, the pituitary gland per hundred grams of body weight was not significantly different in the two series. The "significant ratio" changed from 3.6 to 1.8 when the data was corrected to a hundred-gram body weight basis (Table II).

In the male group the data again showed the pituitary glands to be slightly smaller (— 2.6 per cent) in the rats exposed to cold. This negative difference became positive (6.4 per cent) when corrections were made for differences in body weight (Table III). No significance is attached to these small differences. Therefore, one may conclude that in both male and female rats the mean pituitary weight was not

significantly altered by exposing the rats to cold. Similarly, the quantity of gonad-stimulating hormone of the hypophysis is apparently normal in the rats exposed to cold. The method of testing was the usual implant method using immature rats as recipients. The mean weights of the ovaries of these recipients, implanted with pituitaries from the controls and from rats exposed to cold, were 17.4 and 17.5 milligrams respectively (Table V). (This compares to a mean ovarian weight of 13.0 milligrams obtained in normal rats of the same litter, Table V).

TABLE V

OVARIAN WEIGHTS IN MILLIGRAMS FOR RATS AT 30 DAYS OF AGE. BOTTOM LINE = CONTROLS. OTHERS RECEIVED ONE MALE PITUITARY GLAND IN THE ABDOMINAL CAVITY AT 25 DAYS OF AGE. TWENTY-FIVE RATS IN EACH GROUP.

Type of Donors	Recipients		
	Range	Median	Mean
Warm Series	9 to 52	1.50	17.4
Cold Series	9 to 60	13.0	17.5
None	9 to 18	13.0	13.0

Thyroid: The thyroid glands were increased in weight in all of the animals exposed to cold, and in all but the 30-day female group this increase was significant. The "significant ratio" was 3.5 for the 15-day group, 2.6 for the 30-day group, and 3.6 for each of the 60-day groups respectively (Tables I and III). When the three female groups were considered as one, the difference in thyroid weight was very striking. The thyroids of the rats exposed to cold were 15 per cent heavier than the controls, and when corrections were made for differences in body weight, they were 20.3 per cent heavier (Table II). These differences in mean weight were also effectively illustrated by the "significant ratio" which was 9.4 before and 12.2 after correcting for the differences in body weight (Table II). It is interesting to note that Sundstroem (1925) reported a decrease in the weight of the thyroid in rats and that Mills (1918) reported a decrease in activity of the thyroid in rabbits due to a warm environmental temperature.

Adrenals: The weight of the adrenal glands was increased in all the groups exposed to cold (Tables I and III), significantly so in the 15-day female and 60-day male groups. The "significant ratio" was 3.5 for the 15-day, 0.7 for the 30-day, 1.8 for the 60-day female, and 3.3 for the 60-day male groups respectively (Tables I and III). When all seventy-five females were grouped together the adrenals of the cold series were 8.3 per cent larger than those of the warm series, which was equivalent to 12.9 per cent when corrections were made for differences in body weight (Table II). A similar comparison in the male group

showed that the adrenals of the cold series were 15.1 per cent (uncorrected) and 26.0 per cent (corrected) larger than the adrenals of the warm series (Table III).

Brain: The brains, cut from the cord at the level of the foramen magnum, were lighter in weight in all the groups exposed to cold. This difference was significant only for the combined female groups (Table II), where the "significant ratio" was 3.9. This result was possible because the standard deviation was relatively small, as shown by the coefficient of variation, which was only 2.3 and 2.6 for the warm and cold series respectively (Table II). Actually the brains were nearly identical in weight, as shown by a mean difference of only 1.5 per cent (Table II). This is further illustrated in Table II by the fact that, after corrections were made for differences in body weight, the brains of rats exposed to cold were 2.6 per cent heavier than the controls, a difference which again was small but significant. Thus we have illustrated here, with the brain of the female rat, a peculiar phenomenon not noted in any of the other organs: namely, that the brain of the rat exposed to cold was significantly smaller before and significantly larger after corrections were made for differences in body weight.

Heart: The pericardium and large vessels were trimmed from the hearts. The hearts were larger in all the animals exposed to cold and significantly so in the 15- and 30-day groups. The "significant ratio" was 4.4, 4.2, and 1.6 for the 15-, 30-, and 60-day female groups respectively (Table I). When all seventy-five females were considered together the results were as usual very significant, the "significant ratio" being 11.5 before and 15.9 after corrections were made for differences in body weights (Table II). In the case of the male rats, the effects of exposure to cold were less marked than were those in the females. The per cent change was 4.2 (Table III) as contrasted to 14.3, 10.8, and 6.5 per cent in the three female groups as shown in Table I. The "significant ratio" was 1.1 before and 3.5 after corrections were made for differences in body weight (Table III). It is noteworthy that the per cent increase in the size of the heart became progressively smaller as the time exposed to cold was lengthened (Table I). This is interesting in light of the reported finding that the basal metabolic rate of rats increased with increased length of exposure to cold, attaining a maximum in thirty days (Schwabe et. al., 1938).

Lungs: The lungs, including the thoracic portion of the trachea, were slightly heavier in the rats exposed to cold. The mean difference amounted to 13.1, 1.4, 2.5, and 5.4 per cent in the 15-, 30-, 60-day female, and the 60-day male groups respectively (Tables I and III). This difference was significant only in the 15-day group, as shown by a "significant ratio" of 3.0 (Table I). But when the seventy-five females exposed to cold were considered as one group, the lungs were

significantly larger, the "significant ratio" being 5.0 before and 8.5 after correcting for differences in body weight (Table II). The lungs of the males exposed to cold were significantly larger only when corrected for differences in body weight, as shown by the increase of the "significant ratio" from 1.3 to 3.6 (Table III).

Thymus: The thymus gland, like the spleen, seemed to be little affected by exposing the rat to cold in these experiments. In the 15-day female group the thymi were 4.0 per cent larger in the rats exposed to cold while in the 30- and 60-day female groups they were smaller than the controls by 8.5 and 13.6 per cent respectively (Table I); but in the males no difference was noted, the means of the two series being nearly identical (Table III). When the seventy-five female rats exposed to cold were treated as one sample, the thymi were 7.4 per cent smaller, a difference which gave a "significant ratio" of 3.1 (Table II). This significant difference was negated by correcting for differences in body weight. Thus all the data support the thesis that the weight of the thymus gland was practically unaffected by exposing the rat to cold in these experiments.

Intestinal Tract: The intestinal tract from the diaphragm to the pelvic inlet was weighed with the pancreas and mesentery attached. In all of the groups exposed to cold the intestinal tract was heavier. The percentage increase ranged from 13.0 to 25.6 per cent in the female groups (Table I), while in the male group a difference of only 5.2 per cent was found (Table III). These mean differences were significant in all three of the female groups taken separately and also when they were combined (Tables I and II). In contrast to this the mean difference in weight of the intestinal tract of the males was only significant after corrections were made for differences in body weight, the "significant ratio" being 1.2 and 3.2 respectively (Table III). The greater weights of the intestinal tracts in the groups exposed to cold can be explained partly by the greater amount of food that remained in the intestines, seen at autopsy, and perhaps also by a more developed muscle mass necessary to move a larger bulk of food. The animals exposed to cold ate approximately thirty per cent more food per day than the controls or warm series. This we have previously discussed (Schwabe et. al., 1938).

Liver: Exposing the rats to cold had very little effect on the liver. In the 60-day female group there was a slight decrease in weight which amounted to 2.8 per cent while all other groups showed an increase ranging from 2.8 per cent in the male group to 10.0 per cent in the 15-day female group(Tables I and III). With the exception of this 15-day female group, where the "significant ratio" was 3.3, the mean weights of the livers were not significantly affected by exposing the

TABLE VI

THE COEFFICIENTS OF CORRELATION (R) OF VARIOUS MEASUREMENTS AND WEIGHTS OF ORGANS, ARE SHOWN FOR ALL THE POSSIBLE COMPARISONS BETWEEN THE WARM SERIES (CONTROLS) AND THE COLD SERIES. IN EACH SQUARE THE "R" OF THE WARM SERIES IS GIVEN FIRST WITH ITS STANDARD ERROR, BELOW THIS IS THE "R" FOR THE COLD SERIES AND A FIGURE WHICH IS THE DIFFERENCE BETWEEN THE TWO CORRELATIONS DIVIDED BY THE STANDARD ERROR. IF THE TWO COEFFICIENTS DIFFER BY MORE THAN THREE TIMES THE STANDARD ERROR OF THE COEFFICIENT OF CORRELATION OF THE CONTROLS THAT DIFFERENCE CANNOT BE ATTRIBUTED TO CHANCE. EXAMPLE: THE "R" BETWEEN THE INTESTINES AND LIVER FOR THE WARM SERIES IS +.79, ITS STANDARD ERROR IS ±.043. THE "R" FOR THE COLD SERIES IS +.94 AND THE DIFFERENCE BETWEEN THE TWO COEFFICIENTS OF CORRELATION DIVIDED BY .043 EQUALS 3.5. THE MEANS (CONTROLS ABOVE) WITH THEIR STANDARD ERRORS ARE SHOWN AT THE BOTTOM OF THE TABLE. SEVENTY-FIVE FEMALE RATS IN EACH SERIES.

$$\text{STANDARD ERROR OF "R"} = \frac{1 - r^2}{\sqrt{N}}$$

	Body	Carcase	Nose to Anus	Anus to Tail Tip	Nose to Tail Tip	Diameter of Tail	Kidney	Spleen	Ovaries	Uterus	Pituitary	Thyroid	Adrenals	Brain	Heart	Lungs	Thymus	Intestinal Tract	Liver
Body		+.991 .002 +.90 89.0*	+.752 .051 +.01 1.2	+.572 .078 +.43 1.8	+.662 .080 +.82 11.1*	+.572 .078 +.42 1.9	+.742 .065 +.81 1.8	-.342 .102 -.08 2.5	+.532 .083 +.27 3.1*	+.302 .106 +.34 0.4	+.962 .030 +.73 4.3*	+.041 .115 +.67 3.7*	+.482 .089 +.36 1.3	+.522 .084 +.10 5.0*	+.562 .079 +.38 2.3	+.542 .082 +.14 4.9*	+.432 .094 +.19 2.6	+.702 .068 +.98 4.4*	+.572 .029 +.72 5.1*
Carcase	+.991 .002 +.90 89.0*		+.734 .054 +.76 0.6	+.552 .081 +.37 0.2	+.722 .064 +.84 5.6*	+.593 .075 +.35 3.2*	+.824 .053 +.95 0.0	-.304 .105 -.26 0.4	+.372 .099 +.50 1.2	+.302 .106 +.34 0.4	+.962 .030 +.71 25.2*	+.012 .116 +.55 4.7*	+.472 .090 +.20 2.5	+.522 .084 +.23 2.9	+.582 .079 +.42 4.2*	+.542 .082 +.24 3.2*	+.442 .094 +.11 4.6*	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Nose to Anus	+.752 .051 +.01 1.2	+.734 .054 +.76 0.6		+.492 .089 +.33 2.6	+.562 .079 +.52 0.5	+.542 .082 +.24 2.7	+.672 .063 +.21 0.6	-.342 .102 -.33 0.3	+.502 .088 +.30 2.8	+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.662 .092 +.27 2.0	+.672 .063 +.45 1.1
Anus to Tail Tip	+.572 .078 +.43 1.8	+.552 .081 +.37 0.2	+.492 .089 +.33 2.6		+.562 .079 +.52 0.5	+.542 .082 +.24 2.7	+.672 .063 +.21 0.6	-.342 .102 -.33 0.3	+.502 .088 +.30 2.8	+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.662 .092 +.27 2.0	+.672 .063 +.45 1.1
Nose to Tail Tip	+.662 .080 +.82 11.1*	+.722 .064 +.84 5.6*	+.562 .079 +.52 0.5	+.562 .079 +.52 0.5		+.542 .082 +.24 2.7	+.672 .063 +.21 0.6	-.342 .102 -.33 0.3	+.502 .088 +.30 2.8	+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Diameter of Tail	+.572 .078 +.42 1.9	+.593 .075 +.35 3.2*	+.542 .082 +.24 2.7	+.542 .082 +.24 2.7	+.542 .082 +.24 2.7		+.672 .063 +.21 0.6	-.342 .102 -.33 0.3	+.502 .088 +.30 2.8	+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Kidney	+.742 .065 +.81 1.8	+.824 .053 +.95 0.0	+.672 .063 +.21 0.6	+.672 .063 +.21 0.6	+.672 .063 +.21 0.6	+.672 .063 +.21 0.6		-.342 .102 -.33 0.3	+.502 .088 +.30 2.8	+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Spleen	-.342 .102 -.08 2.5	-.304 .105 -.26 0.4	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3		-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3	-.342 .102 -.33 0.3
Ovaries	+.532 .083 +.27 3.1*	+.372 .099 +.50 1.2	+.472 .090 +.20 0.6	+.502 .088 +.30 2.8	+.502 .088 +.30 2.8	+.502 .088 +.30 2.8	+.502 .088 +.30 2.8	-.342 .102 -.33 0.3		+.242 .109 +.25 0.1	+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Uterus	+.302 .106 +.34 0.4	+.432 .094 +.56 1.4	+.282 .108 +.56 2.6	+.242 .109 +.21 0.6	+.242 .109 +.21 0.6	+.242 .109 +.21 0.6	+.242 .109 +.21 0.6	-.162 .114 -.33 0.3	+.242 .109 +.25 0.1		+.732 .054 +.58 6.4*	+.672 .063 +.76 1.1	+.482 .089 +.50 0.3	+.492 .089 +.23 2.9	+.582 .079 +.12 5.6*	+.542 .082 +.09 5.5*	+.442 .094 +.19 2.6	+.722 .064 +.78 1.1	+.812 .029 +.70 1.9
Pituitary	+.962 .030 +.73 4.3*	+.962 .030 +.71 25.2*	+.642 .068 +.50 2.0	+.822 .084 +.33 2.2	+.822 .084 +.33 2.2	+.732 .054 +.58 6.4*	+.962 .079 +.25 8.9*	+.792 .043 +.68 2.6	-.282 .108 -.14 1.1	+.672 .063 +.76 1.1	+.602 .074 +.52 1.0								
Thyroid	+.041 .115 +.67 3.7*	+.012 .116 +.55 4.7*	+.032 .115 +.51 4.1*	+.032 .115 +.41 2.8	+.072 .115 +.37 2.6	+.072 .115 +.37 2.6	+.072 .115 +.16 0.9	+.072 .115 +.32 2.1	-.042 .115 -.08 3.1 *	+.042 .115 +.48 4.2*	+.162 .112 +.59 4.8 *	+.162 .112 +.48 2.0							
Adrenals	+.482 .089 +.36 1.3	+.472 .090 +.20 2.5	+.382 .099 +.25 1.3	+.342 .102 +.03 3.1*	+.512 .085 +.20 3.7*	+.162 .113 +.09 0.6	+.642 .065 +.52 5.3*	-.162 .112 +.09 2.4	+.762 .049 +.64 2.4	+.482 .089 +.50 0.3	+.562 .081 +.47 1.0	+.412 .098 +.34 0.2							
Brain	+.522 .084 +.10 5.0*	+.522 .084 +.10 4.9*	+.492 .089 +.23 2.9	+.312 .105 +.32 0.1	+.482 .089 +.30 2.0	+.192 .111 +.04 1.4	+.622 .072 +.03 5.1*	-.322 .106 -.46 1.4	+.542 .099 +.28 1.4	+.162 .112 +.36 1.6	+.402 .097 -.04 8.7*	+.112 .114 +.17 0.5	+.392 .098 +.01 5.9*						
Heart	+.562 .079 +.38 2.3	+.582 .082 +.42 4.2*	+.482 .089 +.11 4.6*	+.382 .099 +.10 2.0	+.562 .079 +.12 5.6*	+.372 .099 +.28 0.9	+.762 .065 +.42 6.1*	-.072 .115 +.21 2.4	+.612 .096 +.37 0.4	+.082 .116 +.12 0.8	+.842 .082 +.40 1.7	+.082 .115 +.26 1.7	+.512 .085 +.54 0.4	+.582 .076 +.04 7.1*					
Lungs	+.542 .082 +.14 4.9*	+.582 .075 +.35 3.2*	+.512 .086 +.08 5.8*	+.382 .099 +.17 2.1	+.542 .082 +.09 5.5*	+.392 .099 +.24 1.7	+.682 .092 +.54 1.0	-.082 .115 +.25 2.0	+.672 .090 +.39 0.9	+.212 .110 +.03 1.7	+.632 .070 +.18 6.3*	+.112 .114 +.36 2.1	+.372 .099 +.01 3.6*	+.442 .093 +.16 3.0*	+.562 .079 +.45 1.4				
Thymus	+.432 .094 +.19 2.6	+.392 .098 +.37 0.2	+.362 .101 +.16 2.0	+.272 .107 +.27 0.0	+.662 .091 +.18 2.1*	+.372 .099 +.20 1.7	+.142 .113 +.14 0.0	-.112 .114 +.08 0.9	+.072 .115 +.50 3.5*	+.232 .109 +.27 0.3	+.412 .096 +.19 2.7	+.242 .109 +.46 2.1	+.212 .110 +.04 1.8	+.182 .112 +.15 0.3	+.192 .111 +.39 1.5	+.512 .086 +.55 0.5			
Intestinal Tract	+.702 .068 +.98 4.4*	+.822 .056 +.78 1.1	+.542 .082 +.70 1.9	+.272 .096 +.22 1.5	+.542 .082 +.45 1.1	+.662 .092 +.27 2.0	+.722 .056 +.72 0.0	-.342 .109 -.16 0.7	+.222 .110 +.38 1.1	+.184 .113 +.20 0.4	+.702 .069 +.59 1.9	+.212 .110 +.36 1.8	+.222 .110 +.49 2.3	+.322 .106 +.04 2.6	+.382 .108 +.40 1.3	+.482 .089 +.31 1.9	+.412 .096 +.09 3.8*		
Liver	+.572 .029 +.72 5.1*	+.812 .039 +.68 3.4*	+.602 .074 +.58 0.3	+.632 .064 +.16 2.9	+.632 .070 +.38 3.8*	+.552 .102 +.22 1.2	+.782 .044 +.76 0.5	-.182 .113 +.26 3.6 *	+.362 .101 +.41 0.6	+.172 .112 +.21 0.4	+.702 .044 +.67 2.7	+.162 .112 +.41 2.2	+.462 .091 +.52 0.6	+.312 .106 +.34 0.2	+.442 .093 +.43 0.1	+.442 .093 +.27 1.8	+.372 .099 +.08 3.0*	+.782 .043 +.96 3.5*	
	Body 1822 2.89 1462 1.78	Carcase 1802 1.54 1112 1.19	Nose to Anus 1782 0.87 1782 0.57	Anus to Tail Tip 1882 0.57 1822 0.46	Nose to Tail Tip 3312 1.04 3282 1.10	Diameter of Tail 7.72 0.06 7.72 0.06	Kidney 1210±15.6 1518±16.5	Spleen 882 19.2 780 17.3	Ovaries 80 0.71 462 0.65	Uterus 3302 8.06 2442 7.50	Pituitary 6.32 0.13 7.32 0.14	Thyroid 10.92 0.09 12.52 0.15	Adrenals 482 0.47 522 0.57	Brain 17072 4.45 16802 5.08	Heart 6232 3.90 6942 4.36	Lungs 10332 9.28 10952 8.47	Thymus 3142 0.98 2912 6.49	Intestinal Tract 142.82 0.30 152.02 0.38	Liver 6782±112.3 5642± 86.5

female rats to cold (Tables I and II). When the seventy-five females were taken as a group and corrections made for differences in body weight, the livers of the rats exposed to cold were 7.6 per cent larger and the "significant ratio" was 3.1 as contrasted to 1.3 before correction (Table II). In the male group the livers of the cold series were 2.8 per cent heavier before and 12.4 per cent heavier after corrections were made for differences in body weight. The "significant ratio" likewise became greater, being 0.7 before and 3.2 after this correction (Table III).

COEFFICIENTS OF CORRELATION

The coefficients of correlation between each measurement or weight of organ and all the other measurements and weights of organs are given in Table VI. The significance of the correlations between the warm series and cold series has been determined according to the amount of difference between the coefficients of correlation of the two series. This difference divided by the standard error of the coefficient of correlation of the warm series gives a figure which, if equal to three or more, may be considered to indicate that some factor other than chance was responsible for the difference in correlation between the two series. In these experiments the only known factor which could induce any change between the two groups was the exposure to cold.

In comparing the coefficients of correlation of the warm series and the cold series, forty-eight out of the total of one hundred and seventy-one differ by three or more times the standard error of the coefficient of correlation of the controls or warm series. These are indicated by an asterisk in Table VI.

The data afforded an opportunity to analyze the measurements and organ weights to see which gave the greatest correlation with all others. For purposes of analysis the coefficients of correlation were placed from the largest to smallest (1-19) for each measurement or organ and compared to all the others. To score the results the figures were added across the page, resulting of course in an abstract number. For example, if the body always ranked first, it would have a score of 19. The spleen was always in last place, but due to the fact that some of the coefficients were tied for a given place, the spleen often ranked below 19 and scored 284 and 268 in the warm series and cold series respectively, instead of 361 which would have been the score if the spleen had always been in 19th place. The ranking of the measurements and organs from greatest to least correlations followed by the actual score obtained for each are for the control series:—(1) carcass, 67, and pituitary, 67; (2) body weight, 70; (3) nose to tail-tip, 95; (4) kidney, 109; (5) liver, 114; (6) nose to anus, 118; (7) lungs, 127; (8) intestinal tract, 135; (9) heart, 139; (10) adrenals, 153; (11) ovaries, 168;

(12) diameter of tail root, 174; (13) tail length, 181; (14) brain, 185; (15) thymus, 194; (16) uterus, 201; (17) thyroid, 254; and (18) spleen, 284. For the rats exposed to cold these are:—(1) carcass, 75; (2) body weight, 91; (3) liver, 107; (4) intestinal tract, 115; (5) kidneys, 116; (6) pituitary, 117; (7) ovaries, 124; (8) thyroid, 128; (9) nose to anus, 134; (10) heart, 160; (11) uterus, 164; (12) nose to tail-tip, 169; (13) adrenals, 174; (14) lungs, 182; (15) tail length, 185; (16) thymus, 187; (17) diameter of tail root, 204; (18) brain, 237 and (19) spleen, 268.

Thus it is evident that the body and carcass weights rank at or near the top in both series and indicate that the correlation between body weight and organ weight is high, as Jackson (1914) had previously noted. The kidney ranks high, being in fourth and fifth place in the warm and cold series respectively. The high correlation between the kidney and other organs is to be expected since Stewart (1921) had suggested it and more recently Hall and MacGregor (1937), in bringing together the literature, have discussed the kidney with special reference to body surface. It is interesting to note that the pituitary gland ranks along with the carcass in first place in the control rats, thus again emphasizing the importance of this gland in the regulation of the growth of other glands and organs. The brain, although less variable than any of the other organs (Table II), is poorly correlated with them and ranks near the bottom in the controls and next to last place in the rats exposed to cold. The spleen, because of its negative correlation (Table VI), ranks at the bottom of the list in both series.

DISCUSSION

As the work was in progress it was evident that the weights of the thyroid, heart, lungs, and uterus were affected by exposure to cold. When the results were all tabulated, not only these organs but many others were found to be significantly changed in weight. These changes have been surprisingly consistent as shown by the fact that they recurred in each group. A summary of the data (200 rats) in Tables I, II, and III reveals the fact that the kidneys, thyroid, adrenals, heart, lungs, intestinal tract, and liver are larger, while the body, carcass, tail, spleen, ovaries, uterus, testes, epididymides, pituitary, thymus, and brain are smaller in the rat exposed to cold. Similar changes in weights with the exception of the thymus and liver are found to occur in all four groups. Therefore neither the length of the experiment (fifteen to sixty days) nor the sex seem to be factors of great importance.

The difference in body weight induced by exposure to cold is a disturbing factor. This is especially true in the cold series for those organs that were smaller. To overcome such a difference in body weight, the

data for the combined groups of females (Table II) and for the males (Table III) are also considered on a hundred-gram body weight basis. This analysis seems to be quite satisfactory. The smaller size of the rats exposed to cold may be inferred from the body measurement. There is no evidence in our data supporting a suggestion that the difference in body weight might be entirely due to the greater amount of fat in the controls; but this does not imply that the controls were not fatter. It seems more likely that emphasis should be placed on growth as well as weight.

The cause of the changes in organ weights is unknown. The thyroid is affected most, but this does not necessarily mean that the secretion is more abundant. In fact, the coefficient of correlation for thyroid weight and basal metabolism (cal./m^2/24 hrs.) was —0.14 ($\pm$0.113) in the controls and —0.29 ($\pm$0.106) in the cold series. While this lack of positive correlation gives evidence that the larger thyroids were not the most active in secretion, it should not be concluded (without support of further evidence) that the thyroid gland therefore played no part in the increased basal metabolism; for as we have previously pointed out (Schwabe et. al., 1938), the cause for the increased basal metabolic rate is not clear. The coefficient of correlation for the thyroid weight and adrenal weight in the controls is +0.41 ($\pm$.096) being the highest correlation obtained with the thyroid in the controls (Table VI). This correlation, as Hammett (1925-1926) previously noted, "supports the idea of a special functional inter-relationship between these two organs".

It is interesting to note that the range and coefficient of variation are similar in the controls and in the rats exposed to cold (Tables I, II, and III).

Whatever the cause of the changes in the weights of the organs may be, one should bear in mind that the body temperature was elevated (Schwabe et. al., 1938); thus indicating that the internal organs were not stimulated by cold *per se*, but either through some substance circulating in the blood or possibly by some factor of neurogenic origin.

SUMMARY

1. The measurements of the body and tail, weights of the body, carcass, and all of the organs are reported for two hundred rats; seventy-five female and twenty-five male controls living in a constant temperature, warm room and the same number of litter-mates living in a cold environment sixteen hours each day. The duration of the experiment was fifteen, thirty, and sixty days.

2. The mean, range, percentage change, "significant ratio", coefficient of variation, and coefficient of correlation are discussed. The data are also analyzed after corrections for differences in body weight have

been made. The three female groups are considered separately and combined as one sample.

3. In the rat exposed to cold the body, carcass, tail, spleen, ovaries, uterus, testes, epididymides, pituitary, and thymus were smaller while the kidneys, thyroid, adrenals, heart, lungs, intestinal tract, and liver were larger. The significance of these changes is described.

Only the mean weight of the intestinal tract was significantly larger in all groups exposed to cold.

The brain was the only organ that was significantly smaller before and significantly larger after corrections were made for differences in body weight.

4. The pituitary gland of the rat exposed to cold was unchanged in its amount of gonad-stimulating hormone.

5. The estrous cycles were definitely inhibited by exposure to cold.

6. Neither the length of the experiment (fifteen to sixty days) nor the sex seem to be factors of great importance.

We are indebted to Martin A. Brumbaugh, Professor of Statistics, for his valuable suggestions regarding the statistical analysis of the data.

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HETEROTRANSPLANTS OF EMBRYONIC MOUSE TISSUES

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I

According to Loeb (1935) heterotransplants of adult mouse thyroid in the rat disappear in 8-10 days; ovaries, muscle, bone and bone marrow become necrotic; and cartilage sometimes persists as long as 20-30 days. Various species acting as hosts differ in the intensity and kind of reactions toward heterotransplants irrespective of their relationship to the grafts. Crisler (1929) described the survival of mouse testicular tissue and even of spermatozoa in transplants to the scrotum of the rat after 5-6 months (see Browman, 1937). Reed (1938), and Reed and Alley (1939) found that hair follicles and pigment differentiated in chorioallantoic grafts of embryonic mouse skin.

In the present study, a large number of different tissues from mouse embryos of the 9-day egg cylinder stage to the day-old mouse were transplanted to the omental bursa of the rabbit for comparison both with the above results and with those secured in a previous study of rabbit and rat embryos (Waterman, 1936). Information was sought regarding the relative survival capacities of embryonic and adult mouse tissues in adult heterotransplants, the effect of age upon survival, and the comparative responses of mouse and rat embryonic tissues to the common host. Grafts were recovered after 4-12 days.

II

Transplants of parts of 9- to 10-day embryos did not survive, while only cartilage and epidermis were found in grafts from the 11-day stage. Areas of advanced disintegration were common (cf. Nicholas and Rudnick, 1933, and Hiraiwa, 1927, 1930, for chick chorioallantoic grafts; and Waterman, 1936, for rabbit omental grafts of young rat embryos). In the case of older embryos, small pieces of organs were transplanted. Eight-day grafts of 12- to 14-day embryonic material contained cartilage, bits of kidney showing both secreting and collecting tubules, epithelial vesicles of various sizes and shapes, lung, epidermis, vesicles of intestine with folded mucosa and thin muscular wall, esophagus, tracheal tissues, membrane bone, hair follicles, small vesi-

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cular otic labyrinths, young skeletal muscle, and degenerate eyes without lenses. Tissue of the central nervous system failed to remain alive.

Only a part of the tissue originally transplanted survived in these cases. The fact that some of the tissues from 12 to 14-day embryos do persist while the same regions from 9 to 10-day embryos do not, shows that tissues of the more advanced stages have acquired certain properties conducive to longer survival. The grafts exhibited various stages of degeneration, lymphocytic and granular cell infiltration, presence of phagocytes gorged with cellular debris, increased activity of the host fibroblasts, extravasated blood, multinucleated giant cells, fat cell formation, etc., phenomena characteristic of heteroplastic embryonic grafts. No host blood vessels could be seen entering the transplants.

A longer graft life for this stage resulted in the disappearance of such tissues as kidney and lung and further differentiation of the supporting tissues and epidermis. A few mitotic figures were noted. Cartilage, types of bone, and epidermis were the predominating surviving tissues. The evidences of growth and differentiation, slight as they may be, indicate a certain degree of adaptation as well as a possible utilization of host food material.

Eight-day grafts of material from 15- to 16-day embryos were larger and showed greater survival capacity. The types of surviving tissues were similar to those described for the 12- to 14-day stages. Cartilage nodules, with or without perichondrium, showed scanty intercellular matrix and large, vacuolated lacunae. Numerous precartilage aggregates were present, as well as cartilage and membrane bone. Grafts of older material, including the day-old mouse, gave essentially similar results. Possible exceptions included skeletal elements, skin and whole limb buds. The latter maintained their shape while hair follicles differentiated in the epidermis. No pigment appeared (see Reed and Alley, 1939). Eight to 10 days is about the maximum survival time after which only minute bits of cartilage and epidermis are generally found.

These results seem to show that by the fifteenth to sixteenth day of development practically the optimum in survival capacity of many heterotransplanted embryonic mouse tissues is reached, which indicates a time of some significance in development. In all cases organotypic development is suppressed, while histiotypic differentiation continues in the older material transplanted.

III

Comparison of the present results with those of Loeb (1935) seems to show that after the fifteenth day of development embryonic mouse tissues possess approximately the survival capacity within an adult of a

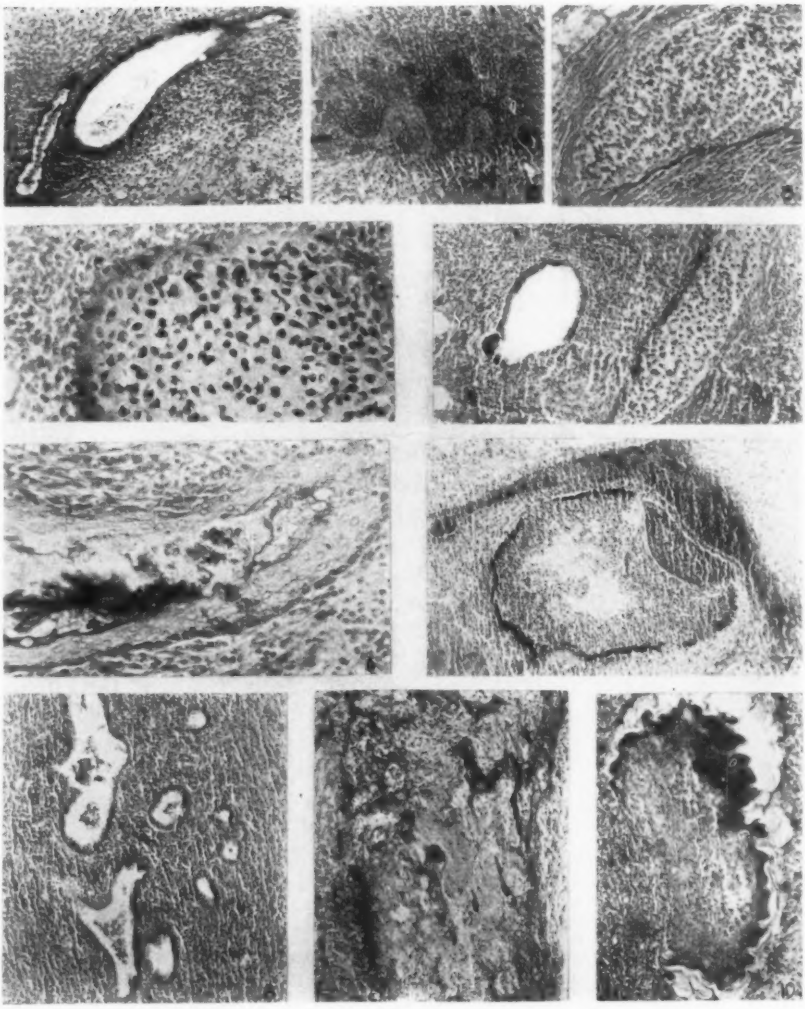

PLATE I

(All figures are photomicrographs)

1, 2, 3. Sections of 4-day grafts of 13-day donor mouse embryonic tissues. x 120. F11. These show trachea and a portion of the lung, epidermis and hyalin cartilage.

4, 5, 6, 7, 8. Sections of 5-day grafts of 13-day donor mouse embryonic tissues. Figures 4 and 6 are x 300; Figures 5. 7, 8 are x 120. F12. These show cartilage, trachea, esophagus, remains of an eye, and lung.

9, 10. Sections of 7-day grafts of 18-day donor mouse embryonic tissues. x 120. F8. These show cartilage and bone, and cornified epidermis.

different species, as do tissues from adults. Any difference may probably be accounted for by the state of tissue organization at the time of transplantation, the older transplants being slightly more resistant to removal. An exception is the work of Crisler (1929) on gonad material. Results seem to indicate that the factors responsible for species specificity (tissue differentials of Loeb) are present early in development and, at the stages tested, are about as active as in adult tissues. Studies of chorioallantoic grafting of mammalian embryos, blood groups in the rabbit embryo (Leeler and Castle, 1933), and reciprocal transplants of rabbit and rat embryonic tissues to the adult (Waterman, 1936) support this conclusion. There is little evidence that the phenomenon of tissue specificity appears suddenly at any developmental stage in either rabbit, rat, or mouse (see Murphy, 1913, 1914 for chick). Nicholas and Rudnick (loc. cit.) did not find any sign of chemical donor-host antagonism between undifferentiated rat embryonic tissues and the chorioallantoic membrane of the chick, apparently indicating the absence of any chemical incompatability.

The cause of failure of mammalian heterotransplants is not clearly understood. Loeb (1935) considers that toxicity of body fluids injures tissues directly and also prevents the establishment of satisfactory connections between the blood vessels of host and transplant. Murphy (1914) states that the cause is due to a refractory condition in the embryos. Loeb and King (1935) speak of "organismal differentials" as being chemical factors whose nature depends upon the genetic constitution of animals. Attempts to reduce the host resistance in mice have not been successful (Browman, 1937). According to Trusler and Cogswell (1935) the failure of homiotransplants in man is due primarily to antagonism between foreign proteins (Blumenthal, 1939). MacFadyen, et. al. (1939) have described the inhibition of transplantable mouse tumor growth by tissue extracts of mouse or rabbit placenta and rabbit or cow mammary gland and their protein fractions.

Comparison of the results of skin transplants with those of Reed and Alley (1939) again emphasizes the fact that an embryonic host, even of a different class, is less antagonistic to heterotransplants than a closely related adult host. While hair follicles differentiated in the omental grafts and the epidermis became cornified, less of the tissue survived and no pigment was formed. Loeb (1935) has reported that various species of adult mammals acting as hosts differ in the intensity and kind of reactions towards heterotransplants irrespective of their relationship to the donor of the graft. However mouse embryonic grafts were quite similar to rat in the rabbit's omental bursa and to rabbit grafts in the adult rat kidney. Survival time was approximately the same as were also the types of surviving tissues and the amount of

further development. As far as could be determined, there was little or no difference in the reaction of the host omental tissues to either rat or mouse embryonic transplants.

SUMMARY

Rabbit omental heterotransplantation of mouse embryonic tissues and organ rudiments of various ages (9-day embryo to day-old mouse) show that: (a) tissues from about the 16-day stage and older possess almost as good survival capacity as certain adult tissues (Loeb); (b) factors responsible for tissue specificity are present in the earliest stages; (c) no conspicuous differences exist in the intensity and kind of reactions or survival between rat and mouse transplants in the common host or of reciprocal embryonic heterotransplants between rabbit and rat; (d) while organotypic development is practically suppressed, histiotypic differentiation may continue in the older material transplanted; (e) it is doubtful if any degree of functional differentiation is attained or long persists.

Tissue from stages younger than 11 days failed to survive. From the eleventh to sixteenth day more transplanted material survived the test period and a progressive advance in differentiation was seen. Little further development was noted in transplants of older material.

Epidermis, cartilage and bone (rabbit and rat) show the longest survival. Epidermis becomes cornified and hair follicles appear. Both cartilage and membrane may develop. Kidney, lung and nervous tissues are quickly removed while others such as the sense organs, gut, muscle, etc. appear to be intermediate as regards survival time.

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MORPHOGENETIC PATTERN ELEMENTS ACCORDING TO THE THEORY OF DIFFERENTIAL PERIODICITY

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INTRODUCTION

This paper brings evidence from morphology bearing upon the theory of differential periodicity, recently advanced by the present writer (Sleggs, 1939). It will be recollected that the theory maintains morphological pattern to be of the same essential nature as the pattern seen when one gauze is viewed through another, the principle being extended to a system of protein lamellae represented, in a state of fragmentation, by the genes in a cellular field.[1] Differentiation is attributed to the direct interaction of genes, whose amino-acids come into varying and recurring alignments (Fig. 1). The absolute nature of the process is not actually involved in the theory: the chemical differentiation of chromosomes could be represented in respect of groupings or by strained valencies which would affect the chemical activity of the gene. In either case it will follow that the alignments pattern will manifest itself in morphogenesis, so that the hypothesis lends itself to examination through comparison between the geometrical properties of an abstract differential periodic system and organic form.

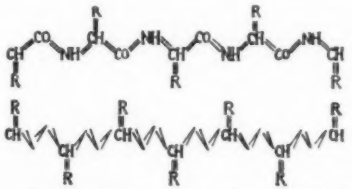

Fig. 1. Protein lamellae in rotational stagger (sectional) R = amino acid residue.

Upon first thoughts it appears that superposed lattices could only give simple geometrical patterns, and it is hard to see how such could be brought into line with the complexity of actual organs. It will be

[1] The production of a cellular field by bifurcating cell lineage does not theoretically give a genar system which could all be combined into a set of protein lattices without altering the alignments. However, the general effect is one of extension of superposed lattices. The theory accounts for proliferation on the basis of such supernumerary portions of the gradient field.

shown, however, that these pattern elements are subject to magnification on an infinitesimal scale, so that by combining them on different scales curves of various types can be produced.

The points which arise in connection with this method of analysis will now be tabulated.

MATHEMATICS

1. *Differential wave*[2] *length.*

a) *Period unequal.* In simple system of linearly superposed units, having spatial periods *a, b,* wave length (i. e., distance between positions of alignment) $= \frac{ab}{a-b}$.

b) *Periods equal, with rotational stagger.* Wave length $= \frac{a \cos \alpha}{1-\cos \alpha}$ where *a* is period and α L. of rotation. For quadrangulated lattices α has the maximum of 45°; for triangulated or hexangulated, 30°.

2. *Form of wave.*

The wave field for all degrees of rotation except in the immediate neighbourhood of the maximum angle, depicts the lattice pattern magnified by the wave length, in which case the value 1 is assigned to the period. Near and at the maximum angle the pattern shows a combination of annularity and radial symmetry (Fig. 4), this configuration being repeated at short intervals which equal the minimum value of the formula. When $\alpha = 0$ (i. e., when the lattices are unrotated) the wave length $= \infty$, meaning that no differentiation results, such a condition probably arising at the points where the spireme breaks into chromosomes (since there is no need for the genes concerned to remain in interaction). The minimum value for the wave length is about $2\frac{1}{2}$ in the case of squared granular layers.

3. *Height of gradient crest* (= maximum degree of alignment).

If $p-q=1$, where $pu=a$, $qu=b$, u being the G. C. M. of *a* and *b*, the gradient crests have equal height (exact alignment). If $p-q=n$, the crests have $\frac{n}{2}$ differences in height, meaning that units come into a degree of alignment which is not exact but higher than that of adjacent units. At the extreme ends of the series, the alignment is exact, but where *a* and *b* are incommensurable this distance is ∞. Giving n the value 2, the wave has alternate sets of crests of unequal height.

Example: coloration of zebra and tiger, in which ghost stripes alternate with dark.

[2] The wave is the curve obtained by plotting degrees of alignment along a straight line.

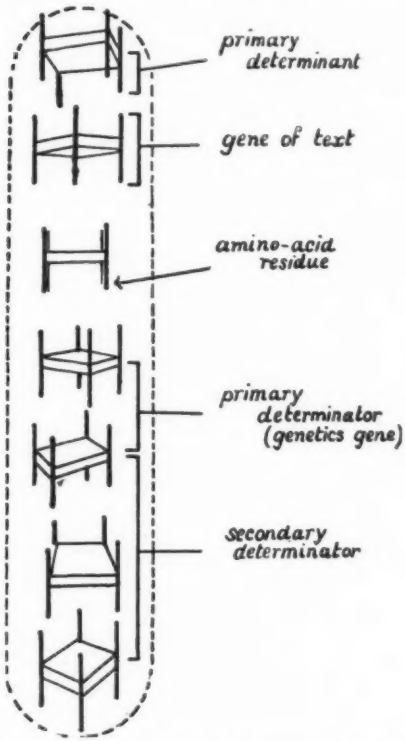

Fig. 2. Diagram of general determinator (chromosome), showing genes in rotational stagger.

DETERMINATION
(Fig. 2)

1. *Determinant* (primary). One of the two interacting faces of two successive genes on the chromosome, with its projecting amino-acid residues.

2. *Determinator* (primary). The two interacting faces of two successive genes.

3. *Secondary determinant.* Gradient crests of primary determinator.

4. *Secondary determinator.* Two interacting gradient fields, each belonging to a primary determinator. Thus, the secondary determinator involves three genes and four half-gene faces.

5. *Nth determinant, determinator.* The same principle is here extended to higher integrations.

It is probable that the so-called unit characters of genetics are compound, i. e., related to determinators of higher order than primary. (If

unit characters were from primary determinants the latter would have to be inclined at an infinitesimal angle to give a wave of sufficient length—an unworkable factor.)

REPETITION OF PARTS

Fig. 3 shows the differentiation pattern produced by a determinator (triangulated), where α=c. 15°. The pattern represents repeated pigmented areas, or, by projection, other recurring structures. If the polygonal mesh of the pattern is considered to represent pigment, the pattern is that frequently seen on the horse.

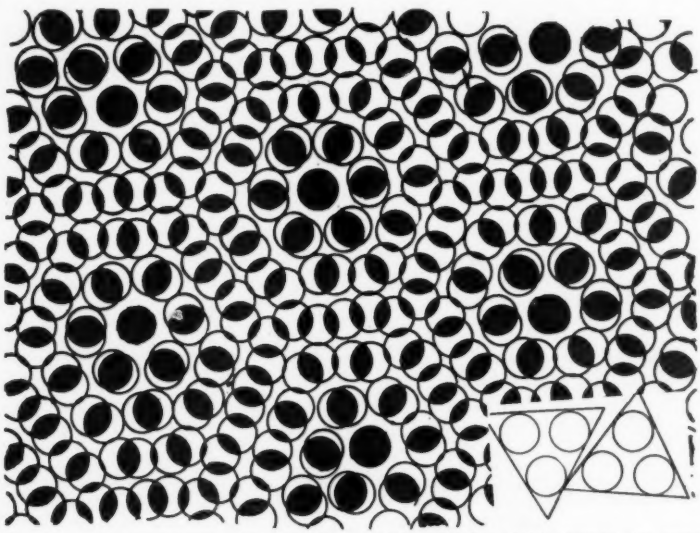

Fig. 3. Harmonic equipotential, or differential periodic, field formed by superposing 2 triangulated lattices at slight angle. Sample of determinator in corner. By blackening overlap, degrees of chemical alignment are represented with general pattern determined by such.

ANNULARITY

In Fig. 4, twelve degrees of stagger have equal value, which is markedly different from the other values. These 12 blocks, (p), have their geometrical centres on a circle, proving that annularity can be expressed in terms of differential periodicity. Examples of annularity are (a) the ocellus, common on the wings of lepidoptera and on feathers, as in peacock, (b) (by projection) layers in emergent structures which do not come to a sharp point. Outpocketings of the embryonic membrane represent annularity, as shown in Fig. 5. Here, the dots represent unit amounts of tissue. By precipitating the invaginant upon the prospective invaginant (magnified to the same scale) and then

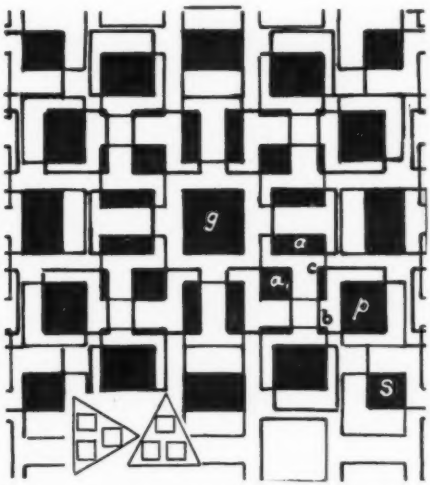

Fig. 4. Field similarly formed, angle of mesh inclination being maximal (30°). Small scale sample of determinator included.

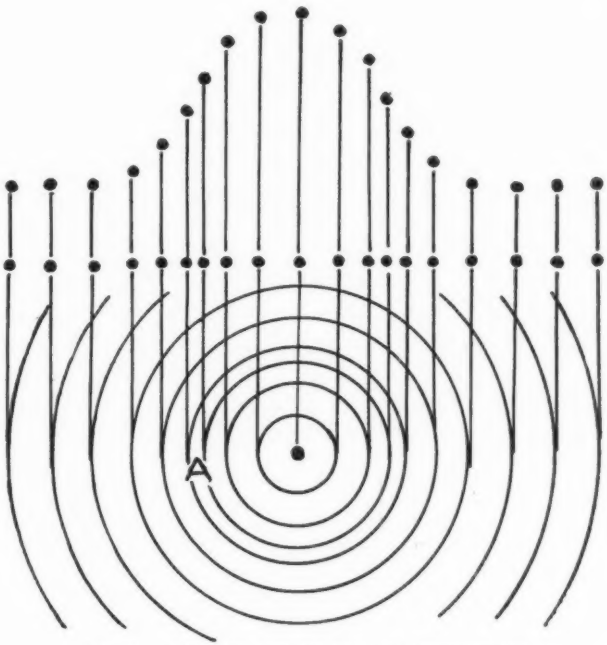

Fig. 5. Showing annular basis of investigation.

continuing the projection lines to depict face view by circles in which amount of tissue is represented by amount of line, a fish-scale-type annulus is formed (i. e., the circles are crowded, at *a*). Hence the prospective invaginant[3] has its growth rate differentiated in an annular pattern.

SYMMETRY

(a) *Radial.* Fig. 4 shows that radial symmetry is reducible to a basis of differential periodicity. It is to be seen that the pattern parallels generalized floral pattern. There is the equivalent of 4 sepals (*s*), 12 petals (*p*), 12 stamens in two groups, say 8 long (*a*) and 4 short (*a*), 1 carpel (*g*), and subsidiary small organs, say nectaries (*b*, *c*).

(b) *Bilateral.* If the determinants, in addition to rotational stagger, have their centres staggered, radial symmetry is converted into bilateral, as shown in Fig. 6, in which the radial floral pattern above is transformed into the zygomorphic, the figure having a resemblance to orchid pattern.

TRIANGULAR ANTITHESIS OF PROPERTIES

(This is the equivalent of the poker-tongs-scuttle relationship whereby combination is inherently adapted to meet wide range of environmental contingencies—poker can cut, tongs grasp, scuttle envelop). Chemically transcribed, an amino-acid residue can be aligned with another, or with the intervening area of the lamella, and intervening area can be aligned with intervening area. It is suggested that the chemical triangle is represented by abrasiveness (molecular strength, from compactness of grouping), adhesiveness (molecular elongation), and slipperiness (molecular flatness). The antithesis manifests itself on various scales of organization. Examples are spine, pedicellaria and tube foot, in sea urchin; transparency, black pigment and visual purple, in the eye. Thus, if *t*, in Fig. 7, represents the perforation for the tube foot, *r* will represent its antithesis, the spine. In the first case circles coincide; in the second, circle falls over interval between circles. This position is surrounded by a radially symmetrical configuration representing partial perforation, corresponding to the flutings on the spine. The third corner of the triangle is represented at *i*, with regions

[3] It has been contended (Pasteels, 1937, supported by Dalcq, 1938) that differential growth rates do not occur in the prefunctional stage of development. As proof of this, data have been produced showing 75 mitoses in 3069 cells in the undifferentiated edge of the trout blastoderm and 3090 in the primitive streak. But these figures only show equality for mixtural frequency: they prove nothing as regards time frequency. Thus, if the ratio $\frac{\text{period of mitosis}}{\text{period of cell life}} = \frac{1}{k}$ the proportion of cells in mitosis is $\frac{1}{k}$ irrespective of rate of division.

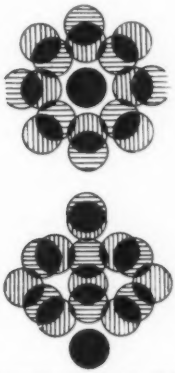

Fig. 6. Derivation of bilateral symmetry from radial, cross-hatched determinant being staggered downwards.

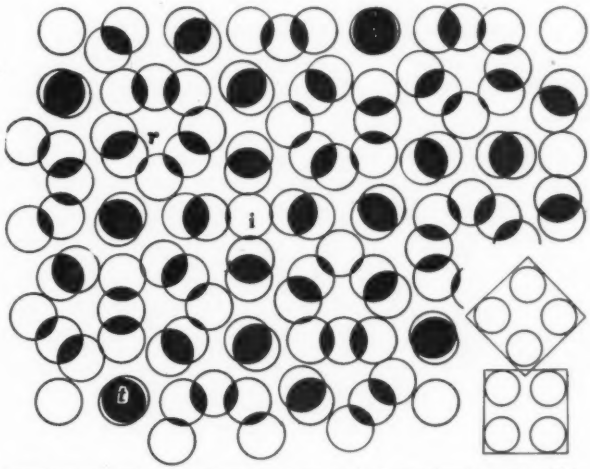

Fig. 7. Quadrangulated determinants staggered at maximal angle, 45°. *t*, *r*, *i* represent triangular antithesis. Small scale sample of determinator in corner.

of sunkenness in a whorl, corresponding to the strong re-entrant angles of the grasping arms of the pedicellaria.

ADDITION OF ORGANS

By a slight adjustment of wave lengths, a number of components can be brought to a summed spike, determining intense local differentiation, whereas the rest of the field shows no substantial alteration of pattern. This explains how it can be that the organism has the power to add some strikingly new structure while retaining its previous pattern. An example is the pitcher of *Nepenthes*, which otherwise is a

typically constructed plant. In the same way certain rodents have highly elaborated anal glands and cheek pouches, absent from related species which otherwise closely resemble the former. Localized sexual demorphism is likewise explicable in cases such as the cat where, apart from the reproductive organs, there is practically no difference between the sexes.

EMBRYOLOGICAL TRANSCRIPTION

The non-differentiation of the egg implies the absence of extensive alignments, the determinator showing statistical uniformity. The extension of the determinator in nuclear division first produces alignments of slight extent. Thus the blastula has a weak annulus in its growth rate, causing invagination. By further extension more extensive alignments are brought about, leading to high differentiation. The egg is thus at a position in the compound gradient field where components interact to produce a statistically flat surface; it is only necessary to proceed laterally to reach crests of varying height. Using analogy, a body of troops marching in natural stride may appear at a given moment to be completely out of step. As they advance certain sets of individuals come into step. Passage along a bridge will produce a differentiated vibration pattern.

The process of differentiation represents the interaction between the determinator and extra-cellular influences, whether from the environment or other cells (hormones). However, the determinator is the main factor since it has a higher degree of organization than the modifier.

APPLICATION TO GENETICS

In a compound wave field a unit character can only be defined subjectively, as a portion of the field whose form can engage the attention of the observer. On this basis the unit characters of genetics will represent, for the most part, summed peaks (chemically: chromosomes with strikingly long alignments or else staircase-like stagger). The absence of a character implies an alteration in the wave length of one or more components so that the crest is dissolved (amino-acids no longer align themselves here).

A number of points will be taken from Morgan (1926) for comment from the standpoint of the present hypothesis.

(1) *Admitted inability of gene theory to explain how gene is related to its end product.* Present theory: product is determined by a region of the extended determinator of which the gene is another region.

(2) *Genes in Drosophila effect several characters at once.* Component wave reinforces or interferes with other components, producing most marked effect where its peak coincides with another.

(3) *Albino guinea pigs have pigmented hairs upon toes—Morgan*

states that this is a difficulty to gene theory. Local growth spurt puts part of skin at greater lineage distance from egg, allowing pigment-determining alignment to arise. Here gradient occurs along line of proliferation.

(4) *Development at high temperature "brings back gene."* Since temperature affects spaces between but not size of atoms, expansion does not preserve exact similitude, degrees of alignment varying. Hence an alignment degree inoperative at temperature x may become operative at $x + a$.

(5) *Mutants frequently revert.* Implies re-alignment of genes.

(6) *Unit characters subject to quantitative variation.* Slight variations of differential (perhaps due to infinitesimal factor) cause failure of crests to sum perfectly.

(7) *Drosophila on acid food has distorted abdomen—not inherited.* Electrolytes, like temperature, distort protein lattices unequally, but alignments will become normal under resumption of normal conditions.

(8) *Overlap between contrasting characters.* Imperfect summing will produce variations in degree of dominance.

(9) *Gene change gives one part specially modified with widespread slight effects.* Explained under "Addition of Organs."

(10) *Reversal of group of genes produces no serious effect.* The orientation of a determinator is immaterial. In such a case only two primary determinators would be disturbed.

SEXUALITY

Genetics can assign no function to sexuality other than that of proffering assortments to selection. This theory (of fortuitous variation) maintains that of the proffered mixture Aa Bb Cc . . . . . n (capitals denoting advantageous variations) the combination ABC . . . . . n can be obtained with sufficient facility to give a workable basis for evolution. The theory, however, fails to discriminate between the effects of altering the value of n, which, when increased to anything beyond a small number renders the fraction of the species to be selected infinitesimal, which is not in accord with what is to be observed in many groups, especially birds and mammals.

According to the present theory, individuals differ in the angles of rotation of their genes so that the interaction of the two determinators in diploidity sets up chemical strain determining increased synthetic activity. Thus the entry of the sperm is followed by sharply increased oxidation, and henceforth the diploid system proceeds along a course of vigorous growth and differentiation, whereas the synthetic powers of the haploid are so feeble that in the vast majority of cases it fails to develop

at all. Correctness is deduced for the view that hybrids are inherently more vigorous and inbreeding deleterious.

The theory of fortuitous variation is mathematically unsound, and must be replaced by one which derives adaptation from the direct interaction between organism and environment. We shall endeavour to show that the properties of a differential periodic system can explain the essential nature of this adjustment.

DISCUSSION

The utilization of rotational stagger for the purpose of deriving these patterns is not a gratuitous postulation but the statement of an inevitable relationship. To object that it may not exist is equivalent to assigning the value zero to the periodicity differential, a special case, the onus of proving which would fall upon the criticism. The fact that the genar material is continually spread out laterally by chromosome division, makes inevitable the conception of a periodicity differential, and hence recurring and variable alignments of chemical groupings. That such must be accompanied by differences in the chemical nature of the cell seems equally inevitable. That the system can give so many explanations of morphological pattern must be regarded as strong presumption that the organism is differentiated on the basis of differential periodicity.

A concrete example of the production of pattern by probable recurring alignment of protein groupings is furnished by the observation of Ramsden (1938) that a striated pattern was formed by shearing coagulation accompanied by the sliding of containing glass plates, in connection with the fibroinogen paste of the silkworm *Bombyx morio*. By grouping lattices drawn on cellophane, using sometimes more than two, we have been able to match the floral diagrams for a number of orders, and also some cases of echinoderm scute pattern. An account of these is reserved in the hope of producing a more extensive set of floral patterns, and for the purpose of working out the mathematical formulae for such patterns. Such formulae will predict the pattern of the chromosome, but with limitations, owing to the fact that there is no means of knowing to what extent the determinants are compound. Theoretically, however, it is possible to dissociate the components, by plotting the determinators for various parts of the whole pattern.

SUMMARY

On the assumption that the chemical nature of the cell will vary according to the alignment pattern of the genes within the chromosome, a series of geometrical patterns are presented representing the interactions of genes which will arise from the fact that the spreading of genar

material through nuclear division will give the general effect of the extension of protein lattices in a state of superposition. It is shown that these patterns can explain repetition of parts, annularity, radial and bilateral symmetry, and the addition of organs without substantial disturbance of previous form. The system gives the theoretical deduction of triangular antithesis of properties, some concrete examples of which are adduced. In connection with annularity, it is demonstrated that invagination and other pocketings of the embryonic membrane can be expressed as the differentiation of growth rate into an annular pattern.

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THE PROCESS OF GROWTH ACCORDING TO THE THEORY OF DIFFERENTIAL PERIODICITY

By

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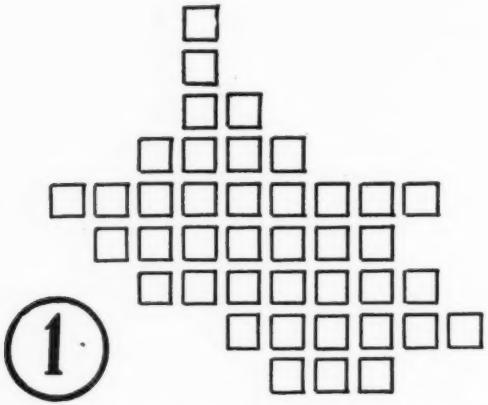
The following paper treats of organic growth in the light of the geometrical principle of organization pointed out in the recent paper (Sleggs, 1939) introducing the theory of differential periodicity, or differential amino-acid frequency, as the concrete form of it might be termed. Whereas the growth of a crystal involves the grouping of unvarying units without restriction as to dimension (Fig. 1), vital growth is lateral extension only, uniformity being restricted to alternate layers (Fig. 2), the differentiation of the intervening layers arising from their structural relation to the first set, which differ in spatial period.

To this type of organization we apply the term *vital mechanism*, since if the system is bent by environmental action the periodicity differentials alter in value, reconstructing the non-uniform layers. Such a peculiar relationship between a body of matter and its environment is, according to the theory, the basis of adaptation. The vital mechanism is in the form of a plate, implying that the organism will be a plate, which is corroborated by the tendency of growth, both in animals and plants, to take the form of extending sheets of cells. In actuality the plate is enclosed upon itself to form a hollow shell†, obvious in animal development and possibly present in obscured form in plants.

The fundamental explanation of the plate form is that a differential periodic system is necessarily restricted to growth parallel to one plane: it is impossible to commingle 3-dimensional lattices of unequal mesh-size since certain units would coincide in position. But when coincidence takes the form of vertical alignment there is no such bar to the existence of the system, and it is free to display its characteristic properties.

Now the system (Fig. 2) appears at first sight (apart from its plate form) to have no connection with any set of established biological facts;

†The origin of the organism is intelligible on the supposition that a protein lattice in the form of a bubble envelops another, a frequency differential in the amino-acids arising from the unequal curvatures. The two lattices now constitute a harmonic equipotential system, to be regarded as living. Sex and life are thus coeval, the first life being non-living in the haploid condition. However, by multiplication of the lattice layers, the system may also become living in the haploid condition.



FIGURES 1 AND 2. ILLUSTRATING ESSENTIAL NATURE OF VITAL GROWTH.
Fig. 1. Crystal growth.

nevertheless it stands in a simple relationship to the organism, as can be appreciated if one introduces the modification of expanding the differentiated layers in the manner shown in Figs. 3, 4, 5. The system is now divided into units, each of which has its determinator in the form of a thread—the otherwise inexplicable peculiarity of a biological cell. The lattices (primordial chromatin) are fragmented into units (genes), and the differentiated layers (primordial cytoplasm) are now represented in the surrounding cytoplasm of the cell which also comes to contain additional water and various secondary materials. The exact nature of the expansion process can be left an open question, but it can be seen that the cytoplasm will contain chemical groupings determined either directly or indirectly by the alignment pattern of the amino-acids within the determinator, represented by the spireme or its chromosomes. The division of the spireme into chromosomes possibly expresses the fact that here and there are two adjacent genes between which the periodicity differential is zero: the association of such could have no effect in differentiation so that there is no need for the thread to remain intact at these points. The determinator will, as the figure shows, have a different stagger pattern from cell to cell. This point would be difficult to establish, but there is evidence that chromosomes actually are internally staggered. Metz and Lawrence (1937) figure such a condition in the giant chromosomes of *Sciara ocellaris*, the chromatin discs overlapping in various degrees. They also refer to the fact that the fine threads which connect the discs "are not arranged linearly, but are staggered". Belling (1926) describes zigzag chromosomes, his observations being regarded by Darlington as perspective effects in relation to spirality. But staggering to right and left, as well as fore and aft, may

result in the appearance of a spiral. The theory is in conformity with Darlington's views in favour of the spiral nature of chromosomes, but it implies that exact spirality is merely a special case of stagger pattern. The continuity of the primordial cytoplasmic layers is preserved in the protoplasmic bridges, whose presence in tissues is generally believed to be universal. The theory implies—and these various suggestions are not sub-hypotheses but deductions from the geometrical properties

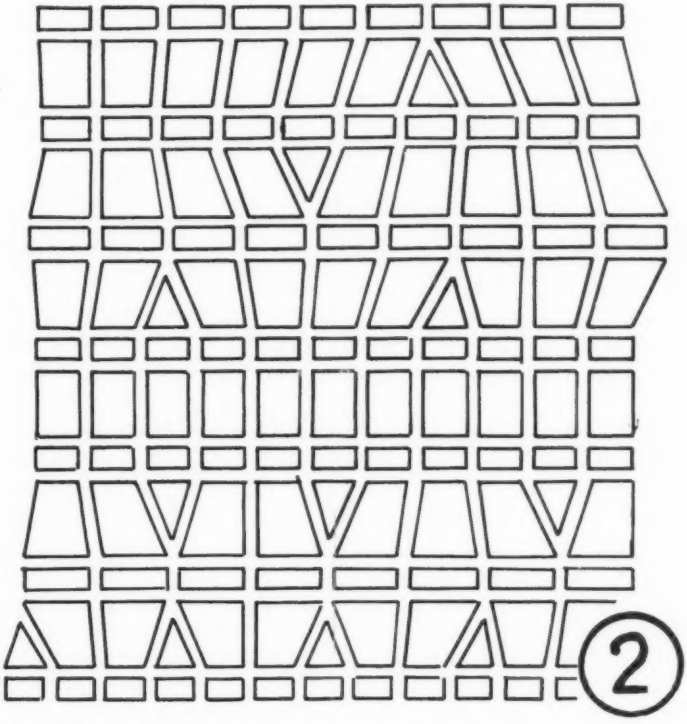
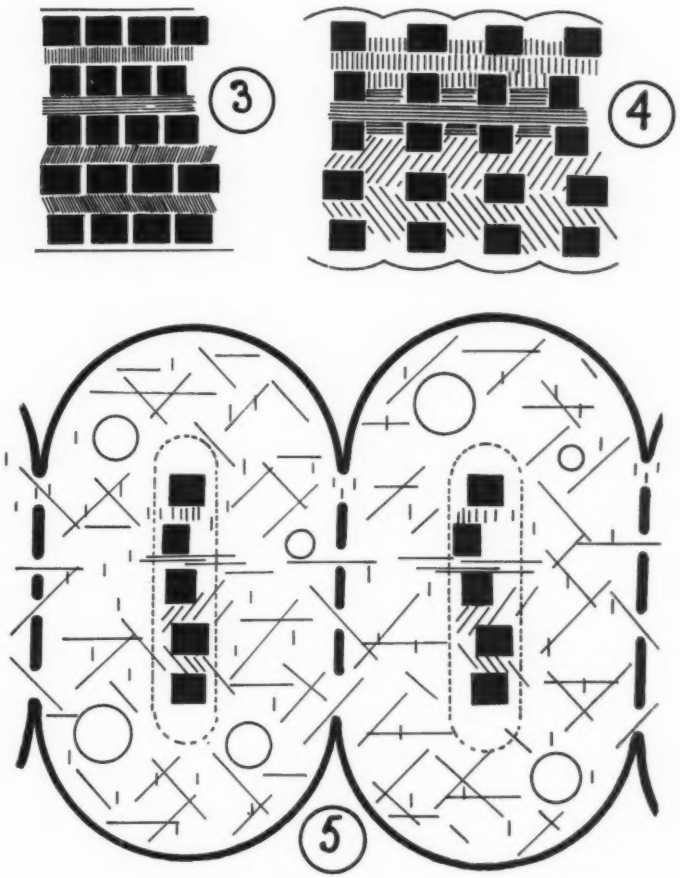


Fig. 2. Differential periodic system or vital mechanism, consisting of uniform (genar) layers, or primordial chromatin, and differentiated layers or primordial cytoplasm. At positions where the stagger gradient changes, a triangle is inserted, denoting either peak or trough of differential wave. The differential wave length is ∞ for the fourth cytoplasmic layer, meaning that the fourth layer is undifferentiated. A position such as this probably marks the division of the spireme into chromosomes, since the two genes involved could produce by their interaction no effect upon differentiation. From the point of view of the theory a spireme is a fragment of this lattice stack running from top to bottom, its width being immaterial. Note that the differential waves can also furnish secondary periodic systems and so on, accounting for the fact that a small number of genes can produce a large number of characters. If periodicity differential is determined by lattice rotation, the diagram is equivalent to a projection of a section of complicated shape.

of the postulated system—that these cytoplasmic connections provide the basis of organizing action. Organizing action, according to the theory, is expressible as lattice slide in the vital mechanism, whereby the position of the differential wave crest is shifted. This process involves continuity of either the genar or the cytoplasmic layers; since the former have lost their continuity, the latter must preserve it.

The transformation of the primordial vital mechanism into the cellular organism is intelligible from the fact that the edges of the cytoplasmic layers will have unequal growth rates, the groupings being of different degrees of obliquity, or chemical strain, and hence having unequal rates of synthesis. A conflict will thus arise between the forces determining enlargement of the system, and those determining coherence. The resultant effect is to divide the system in such a manner as to allow the chromatin to pursue its course of uniform growth step by step with the unequal growth of the cytoplasm. The system is one in which atoms, or groups of atoms, take up their appointed positions with differential facility, the process being complicated by secondary processes connected with the supply of materials and energy. Not only will a cell differentiate internally, but the rate of cell division will depend upon the alignment pattern of the determinator. The basis is thus laid for differential growth rates. According to the theory, neither the chromatin nor the cytoplasm of a cell actually divides, the former extending itself laterally in the way that protein lattices extend. A new determinator, of different alignment pattern having been formed, this now determinates a new cytoplasm, the phenomena of mitosis being the process whereby the two chemical cageworks extricate themselves. Of the two so-called daughter cells, one is the parent. This may "divide" a second time, producing a true sister to the second, both of the latter being identical and different from the parent, which remains in the original condition. It follows from this that the early embryonic cells will remain as such, since any given cell retains its form. This statement, surprising as it may seem, is nevertheless in accord with the fact that embryonic cells do remain in the adult. Their presence, in fact, has often been a difficulty to morphogenetic theory. The process implies, moreover, that the organism will not have every cell different from every other, but will come to contain large groups of identical cells, which is obviously confirmed. Such tissue masses, e. g. muscle, cannot be fitted into the gradient field; hence they are laid down as proliferations of the germ layer, or, as Dürken (1929) expresses it, "in late development there is liberation from interdependence".

At the same time, a correction may have to be introduced into the foregoing. There is reason to think that in organizing the construction of a new cell the parent sustains what might be described as "recoil":



FIGURES 3, 4, AND 5. DERIVATION OF CELLULAR CONDITION. PRIMORDIAL CHROMATIN, OR GENAR SYSTEM, IN SOLID BLACK. INTERNAL DIFFERENTIATION OF PRIMORDIAL CYTOPLASMIC LAYERS IS NOT SHOWN.

Fig. 3. Primordial vital mechanism, or differential periodic system.

Fig. 4. Enlargement of cytoplasmic layers.

Fig. 5. Further enlargement, the condition now representing a unicellular layer. The haploid condition is represented for simplicity, with an undivided determinator, i. e. a single chromosome. The two cells shown correspond to the two right-hand portions of the original stack. Note the different alignment patterns of the genes. The chemical groupings originally determined between the genes are represented as diffusing into the body of the cell where they can interact to produce the characters of the cell. This conception is formal, the exact nature of the expansion being left open. Note the cell bridges, deducible on basis of original continuity. Circles denote secondary inclusions.

that is to say, the new cagework "challenges" the old to construct it in a pattern of greater chemical strain, so that the parent loses pattern in the process. This appears inherently probable, and agrees with Darlington's (1932) statement that of the parent and two daughter cells all three are different. Thus the differential mechanism works with a certain amount of "slip", but as this factor will be accurately determined, the mechanism is still able to execute a precise differentiation pattern, which probably approximates what would be produced were the process rigid. Our conception that development proceeds from lesser to greater chemical strain is based upon the fact that chemical energy increases as differentiation advances (Needham, 1926).

The new cagework having been constructed, the maintenance of its pattern necessitates organizer continuity with the parent; in other words the parent, having produced the new cell, must continue to back it up. Hence in unicellular organisms, and in tissue cultures, where such continuity is lacking, cell division does not lead to differentiation. The multicellular organism is maintained in a state of organizer tension from the fact that it develops as a closed shell, there being no outlet for the strain. The theory suggests that in cancerous tissues the cell bridges will be lacking, since in such tissues the power of differentiation is slight or nil. We do not know whether this is the case. The theory will now be considered in a detailed application.

It is frequently the case that the pigmentation pattern of an animal exhibits disturbance at salient angles in the body, especially snout, ears, tail and feet. The emergent region may bear black or white or both, whilst the adjacent area, or the whole body, may be of neutral colour. Yet no hard and fast correlation can be established between the actual sharpness of an outgrowth and pattern disturbance. In the same animal a blotched emergence may exist along with an unblotched which is even more salient. The correlation, however, is apparent when one considers to what extent the process of growth preserves similitude of form. Certain emergent regions, such as the limbs, grow more or less uniformly with the body, but others, such as horns, arise late in development, or after birth, by a local spurt of growth. In short-horned cattle the horn is usually white, upon a brown or black face. Its epidermal covering is derived from a portion of the epidermal field which was originally coloured and would presumably have remained coloured had the epidermis not been pushed out by the bone. In hornless cattle there is no difference in colour in the position of the horn. The outpushing necessitates the resumption of cell division with the result that the cells of the horn are at a greater lineage-distance from the egg. Hence, the amino-acid alignment which determined pigment in the adjacent areas is overrun in favour of a new alignment which does not have this effect,

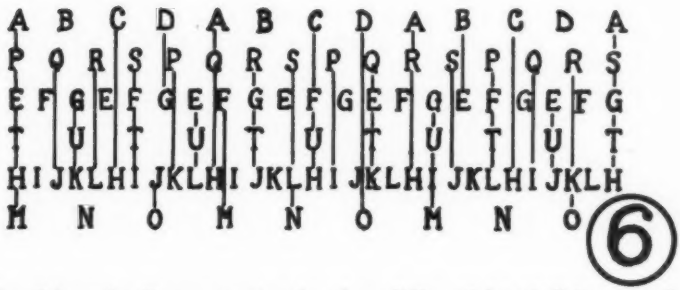


Fig. 6. Schematic alignment system based on differential periodicity. As the system grows, its edge changes in character and can exert differential effects. In this way the extension of the genar lattices in cell division can determine new types of cytoplasm. Note how alignments recur, at distances representing the differential wave length explaining repeated structures.

accounting for the lack of pigment upon the horn. If, however, the horn grows to a greater length, as in long-horned cattle and buffaloes, the original alignment may be restored from the fact that at this still greater lineage-distance a complete differential wave has been traversed. Hence such long horns are white at the base but regain pigment towards the extremity. In cases of still more pronounced outgrowths, several waves may be traversed, producing a banded condition; an example is the tail of the lemur in which the great length is attained entirely after birth. The spines of the hedgehog and porcupine are banded and it would seem certain that these develop mainly after birth. The most pronounced local growth spurts known are represented in the feathers of birds and the wings of lepidoptera, and these are significantly the most highly patterned structures known. Evidence from pathology confirms the same point of view. Skin carcinomata in white horses are melanotic, the resumption of cell division having produced the pigment-determining alignment which is not attained in the normal skin. In the same way we can account for the fact—which is a difficulty to Morgan (1926)—that albino guinea pigs have a few pigmented hairs upon the feet. We agree with Morgan that the gene is not present-or-absent but changes, but the change is merely one of position. The gene either is, or is not, so adjusted in the egg that by the time the skin is reached it will be aligned to determine pigment. Since it is always present, all individuals can produce pigment if their cells run to the right lineage-distance from the egg.

Now, since organs which appear late in development will represent recent evolutionary acquisitions, it follows that specific peculiarities of form will be accompanied by local pigmentation disturbance. We have confirmed this by a systematic survey of museum collections.

It might be objected that the theory implies identity of form for cells at equal lineage-distance from the egg, which would give an insufficient number of cell types. Assuming that the egg doubles itself regularly, the number of cells in the adult will be 2^n, and calculation shows that n will be below 50 for the majority of animals. Allowing for inequalities for lineage, and for size, it seems safe to say that even in the largest animals n will never exceed a few hundred, which would only give that number of cell types. The answer to this difficulty is that in all stages of development, until proliferation of differentiated cells occurs, the embryonic membrane is continually reorganizing itself whereby the bifurcating line of cell lineage is integrated laterally into a continuous differential stack, possessing length and breadth. The argument so far, has only dealt with the differential as it arises along a cell line. In addition to this complication, a new set of differentials arise out of the curvatures of the embryonic membrane, introducing a further adjustment need in the organizing process. The number of possible cell types is thus very great. The advantage of the evidence just introduced is that it concerns a department of growth where the complication from the organizer is largely or wholly absent, in the sense that the growth is now in Dürken's region of liberation from interdependence. We originally thought that the disturbance in pigmentation was the result of differentials associated with curvature, but a closer study showed that this could not be so, and that the facts presented an ideal example of straightforward realignment action.

The theory implies that the only essentially vital activity in the organism is organizing action, though this may be obscured by secondary processes. Nerve and muscle action are the organizer operating at high speed. It allows only of two ways in which the organism may come into adjustment with its environment: by shifting the differential wave crest and by altering the differential wave length (or its reciprocal, the periodicity differential). We derive the periodicity differential from rotation of genes, rather than chemical variety among the genes; the considerations for this must be deferred to a later paper. Expressing this for an individual cell, an environmental influence—whether from the outside or from another part of the body—either slides the gene transversely or else rotates it, resulting in new amino-acid alignments via a new cytoplasmic framework now in equilibrium with the influence, whether it represents a gross mechanical force, or the action of light, or the intrusion of a chemical. Diffusible evocating substances, as in the optic cup which transforms an implant into lens, act by their power to determine a specific alignment pattern. The case of hormones is likewise intelligible; their differential effects are due to a differential component in the interaction in that the pattern to be altered varies.

The final form of the organism represents an interaction between the egg and its environment, the former being the preponderating factor since the interaction is between two systems of unequal degrees of organization.

MATHEMATICS OF THEORY

Like most theories in organic chemistry, the present one is non-mathematical in the sense that it postulates a certain arrangement of units which can be stated without numerical data. At the same time this arrangement embodies a mathematical principle, viz. differential periodicity, which permits of comparison between deducible and actual patterns. The theory is essentially an abstract conception, and preserves a certain independence of concrete hypothesis. It would be open to any one else to suggest other substances than proteins as the determinants: all that is needed is a cagework with lattices of recurring structures separated by structurally related grouping. In the abstract system, the differential wave length $= \frac{pq}{p-q}$ where p and q are the lengths of the interacting units.

If $a - b = 1$, where au is the width of one unit and bu of the other, u being the Greatest Common Measure of au and bu, the differential wave has all its crests of equal height. If $a - b = 2$, the wave has alternate crests of unequal height. Such a wave may be exemplified in the ghost stripes of the zebra, the pigmentation gradient rising high to produce the dark stripes and low to produce the faint stripes in the intervening areas. The mathematical treatment would be a formality, since the properties of the system can be ascertained from geometrical diagrams. The concrete expression of a differential unit is the distance between two recurring amino-acids in the protein lattice; where the lattices are rotated these two may not be the nearest two, but two on a line running obliquely through the lattice, i. e. through homologous genes along the line of cell lineage.

The *differential gradient*—which is the same as the gradient in Child's theory—is expressible in terms of lattice stagger, which varies from point to point according to the relative periodicity. If two amino-acids are exactly aligned at one point in the lattice stack, the corresponding two will be staggered in the daughter cell, the stagger increasing and then declining, (from the fact that a unit is now related to the next opposite one) to another position of exact alignment when more genes are laid down in cell division. It is so far arbitrary from the point of view of the theory as to whether the gradient crest is to be identified with maximum or minimum stagger. Our conception, how-

ever, is that the egg exists in a condition of minimum stagger, associated with its undifferentiated character. The sperm, on the other hand, being a highly differentiated cell, has its determinator highly staggered. When it enters the egg its determinator collapses to the position of minimum strain, corresponding to that of the egg, so that the fertilized egg is little different in its structure from the unfertilized. This collapse involves lateral shift of the genes, but not rotation, the essential of sexual reproduction being the commingling of characters determined by genes rotated according to slightly different patterns. The organizing process continually going on in the life of the individual involves the germ cells, so that these rotational patterns have been acquired, from the interaction of the mechanism with its environment. The significance of sexual reproduction can be expressed by the provisional analogy of a stereoscope, allowing the vital mechanism, as it were, to look at its environment with two eyes instead of one, sex involving an improvement in the power of adaptation.

DISCUSSION

The theory establishes a connection between equipotentiality, gradient, membranous growth, and determinator in the form of a thread. It gives a system in which differentiality does not lead to persistent divergence, but to divergence followed by convergence, and so on in a cycle. It integrates the conceptions of preformation-by-corpuscles and epigenesis, i. e. the organization of higher states of complexity. As far as we know, all biologists, from the time of Weismann, have maintained that these two doctrines are incompatible. Weismann (1893) wrote: "I tried in several ways to arrive at a satisfactory epigenetic theory, which, starting with a germ substance of comparatively simple structure, should exhibit differentiation as due to regular changes brought about by division of the primary structure. But the more I considered the matter, the more I was convinced that such a solution was impossible". Weismann's error lay in dividing his determinator; had he extended it by fitting units to the edge, he might have seen that the impossibility did not exist. A generation later, Dürken (1929), upholding epigenesis, continues to agree with Weismann that the two concepts cannot be reconciled. But differential periodicity effects this integration from the simple deduction that certain alignments within the determinator can vary the rate of cell division and hence the rate at which new alignments are brought into play to vary it still further. Upon one rate it superimposes another, and also rate of change of rate. Expressed alternatively, the differential wave between two adjacent genes, can establish, with the other waves, differential waves of higher order. This is epigenesis, and yet it exists

upon a corpuscular basis. Differentiation amounts to the expansion of a mathematical function.

The organism is merely a unique kind of mechanism. Its properties appear non-mechanistic because such a mechanism is unknown among other systems, either natural or artificial. "The organismic faculty", (Dürken), "which cannot be referred to any organization either mechanistically or morphologically explicable", is dissolved.

SUMMARY

The paper presents a concept of the essential nature of organic growth as compared with crystal growth. The former is restricted to lateral extension, alternate layers remaining uniform, the intervening being differentiated from their structural relation to the first set which differ in spatial period. The uniform layers are the genar system or primordial chromatin, the differentiated layers the primordial cytoplasm. By expansion of the latter the genar stack is divided into threads surrounded by cytoplasmic masses; hence a cell has its determinator in the form of a thread. The determinator has its amino-acids aligned according to a variety of patterns, determining differential rates of synthesis. Differential growth rates are expressible basically as the rates at which atoms fall into position in a chemical cagework which extends in the manner described. The alignments follow one another in succession according to the geometrical properties of the differential periodic system. The alignment in one cell, by affecting rate of division, also affects rate at which new alignments are brought into play. Hence one growth rate determines a succeeding one and also the rate of change of rate. The theory thus places epigenesis upon a corpuscular basis, hitherto maintained impossible.

The theory is next applied to interpret the disturbance of pigmentation pattern in connection with salient parts of the body. No satisfactory correlation can be established between the actual sharpness of an emergent structure (horn, ear, snout, tail, limb, feather, etc.) and the presence of blotching upon it. The correlation is restricted to cases where the saliency arises from a local growth spurt. The interpretation is that the local addition of cells produces determinators at a greater lineage-distance from the egg and hence with new alignments determining absence of pigmentation or deeper pigmentation or both. It follows that specific peculiarities of form, irrespective of the absolute nature of the contours, will be accompanied by local pattern disturbance. This is maintained to be the case, based on surveys of museum collections.

APPENDIX

Chemical Strain

A chemical cagework in the form of a differential periodic system exists in a condition of chemical strain, i. e., valencies are tilted from the normal angles. This produces high synthetic activity, which is characteristic of living matter (since molecular vibration, with the consequent opportunities for combination, is increased). When sugars, fats, etc., which, outside the organism are oxidized slowly, or not at all, are incorporated in the strained system they are rendered capable of high chemical activity, accounting for the power of living matter to obtain energy in a degree not possible in non-vital systems. Thus, growth is the extension of matter under sustained chemical strain up to a point where the energy potential cannot drive the process any further; here differentiation stops. Hence life cannot normally be suspended, since cessation of the flow of energy will allow reorganization of the cagework to a non-periodic or inanimate condition.

Intracellular Differentiation

The proteins, etc., of the cytoplasm, will interact among themselves to form subsidiary periodic systems, allowing the cytoplasm to differentiate internally, the pattern often resembling in miniature that of a cellular organism. In the latter the genar system represents a differential periodic system of a higher order, which coordinates the properties of the subsidiary systems.

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THE EFFECT OF INCREASED ATMOSPHERIC PRESSURE ON THE ALLANTOIC VASCULAR BED AND THE BLOOD PICTURE OF THE DEVELOPING CHICK

By

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(*Received for publication January 15, 1940*)

Earlier investigations (Cunningham, 1936) have shown that increased atmospheric pressure accelerates the early developmental rate of chick embryos, but that in such eggs there is a high mortality at about eleven days of incubation. Superficial examination of the dead embryos suggested that the fatalities were due to a lack of proper development of the circulatory system, especially the extra-embryonic circulation. These experiments were undertaken to clarify this point.

The experimental White Leghorn eggs were incubated at forty pounds pressure for ten days under otherwise standardized conditions, while some of the controls were incubated for ten days and others for eight days under the same standard conditions but at atmospheric pressure.

At the end of the incubation period an opening was made into the air space and the embryo manipulated so as to expose the allantoic vessels. Blood was then drawn for examination. Heparin was used to prevent clotting. The vessels were then injected with thorotrast for ten minutes at a gravitational pressure of 6 cm. after which the air space was filled with melted paraffin.

The eggs then were X-ray photographed* in three positions. One picture was made from the air space end; a second from the top and another after the egg had been rotated through about 180°. The pictures of all eggs used in the experiment are shown in the plates. There has been no selection of specimens.

From the evidence it would appear that the allantoic vessels are much less developed in the ten day pressure chick than in the ten day normal, despite a weight increase of 40.0%; in fact, the vessels resemble more those of the normal eight day chick.

The blood was subjected to routine investigation for cell counts, cell size and hemoglobin content. The results are shown in the table.

*The authors wish to express their appreciation to the X-ray department of Duke Hospital for making the negatives.

	8 Day Controls	10 Day Experimentals	10 Day Controls
Red blood cells:			
No./cu. mm.	1,210,000	860,000	1,880,000
Size (avg. diam.)	10.68μ	11.04μ	10.02μ
Hemoglobin gms./100 cc. blood	9.3	5.9	14.7
White blood cells:			
Lymphocytes	54.0%	39.0%	38.5%
Polymorphonuclear leukocytes:			
Eosinophiles	32.0%	36.0%	36.5%
Basophiles	7.5%	6.0%	7.0%
Neutrophiles	2.0%	14.5%	13.0%
Monocytes	4.5%	4.5%	5.0%

An analysis of the data indicates that the ten day pressure chicks have not reached the red cell count of the eight day controls, and while the cells are larger the hemoglobin content per cell is less. The embryos subjected to ten days incubation under increased pressure offer a blood picture which is more embryonic than that of the eggs incubated under atmospheric pressure for ten days. There seems to have been little, if any, effect on the white cells.

Studies on the effect of increasing the oxygen content of the air surrounding developing hen eggs give some results comparable to those observed under increased atmospheric pressure. There are other observations which seem to indicate that the effects are not wholly comparable. Baudrimont and Martin de St. Ange (1847) using 85% oxygen observed red corpuscles in the amnionic fluid. This was confirmed by Riddle (1921), but denied by Pott and Preyer (1882), and we did not observe it in our material. Riddle as well as Remotti observed many anomalies and a high death rate if the oxygen was increased at or near the beginning of the incubation period. In our more perfect experiments there was neither an increased death rate nor unusual production of monsters, although pressures of 40 pounds were reached within twenty-four hours of the time the eggs were set. Remotti observed that there was a marked reduction of the allantoic network under increased oxygen; this is very evident in our studies of increased atmospheric pressure. Riddle, while he called especial attention to the apparent lack of hemoglobin, evidently did not make studies of the blood picture, such as are reported herein.

The poorly developed extra-embryonic vessels coupled with the low number of red cells and their low hemoglobin content would seem to be a factor in the high mortality among chick embryos incubated under

increased pressure. This retarded development of the respiratory mechanisms might work for the fatality by the constantly increasing oxygen demand of a growing volume of tissue exceeding the limit of supply.

SUMMARY

Although the embryos of eggs incubated under increased atmospheric pressure are 40% heavier than controls of the same age, their allantoic vascular bed is much less extensive and their red blood cells are more embryonic and fewer in number than in the controls of the same age.

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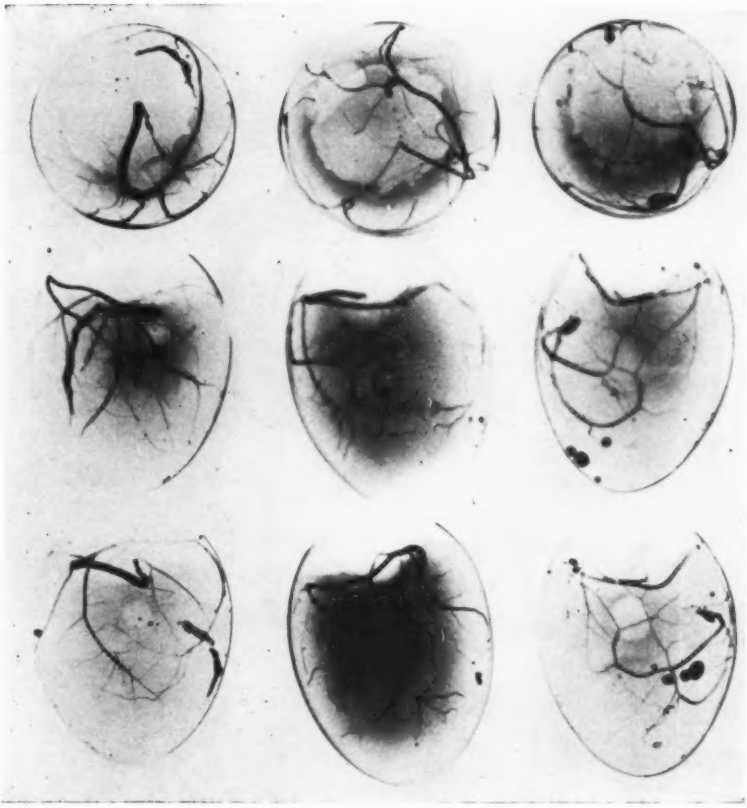
PLATE I

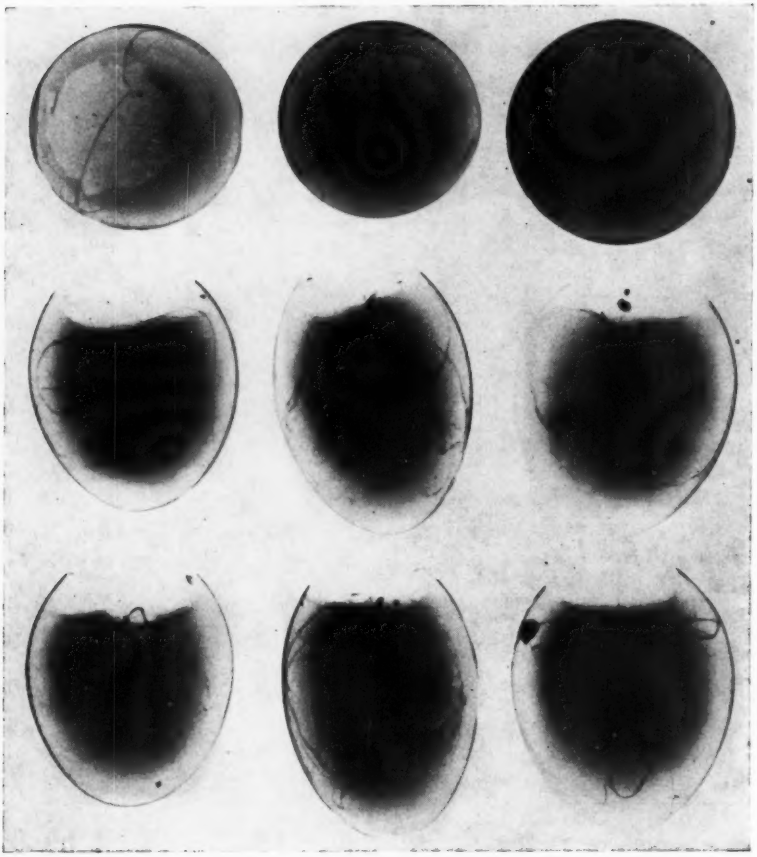
X-ray photographs of three eggs incubated for 10 days at atmospheric pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after approximately 180° rotation.

PLATE II



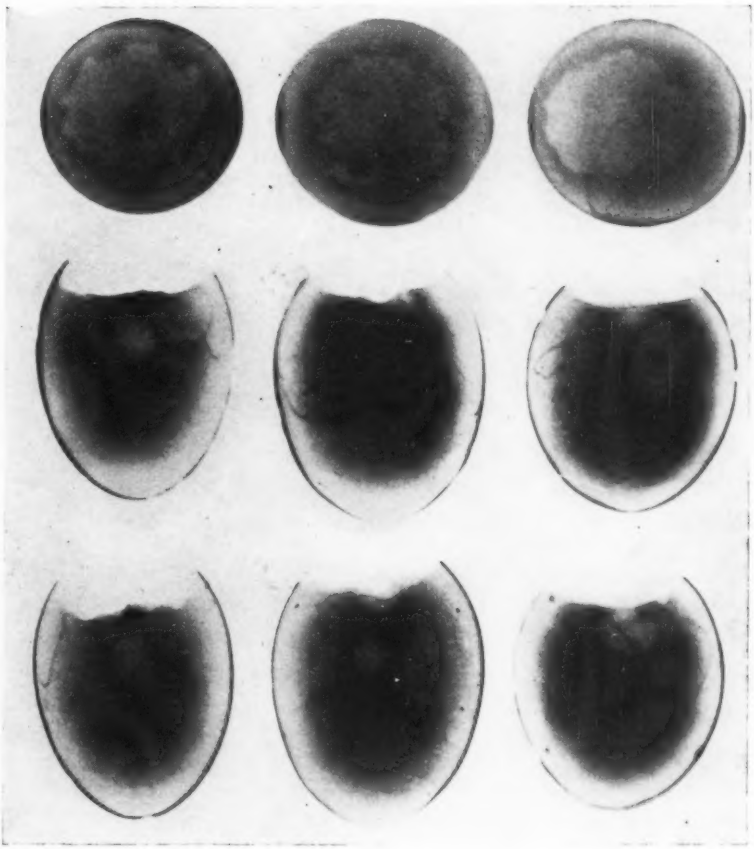
X-ray photographs of three eggs incubated for 10 days at atmospheric pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after approximately 180° rotation.

PLATE III



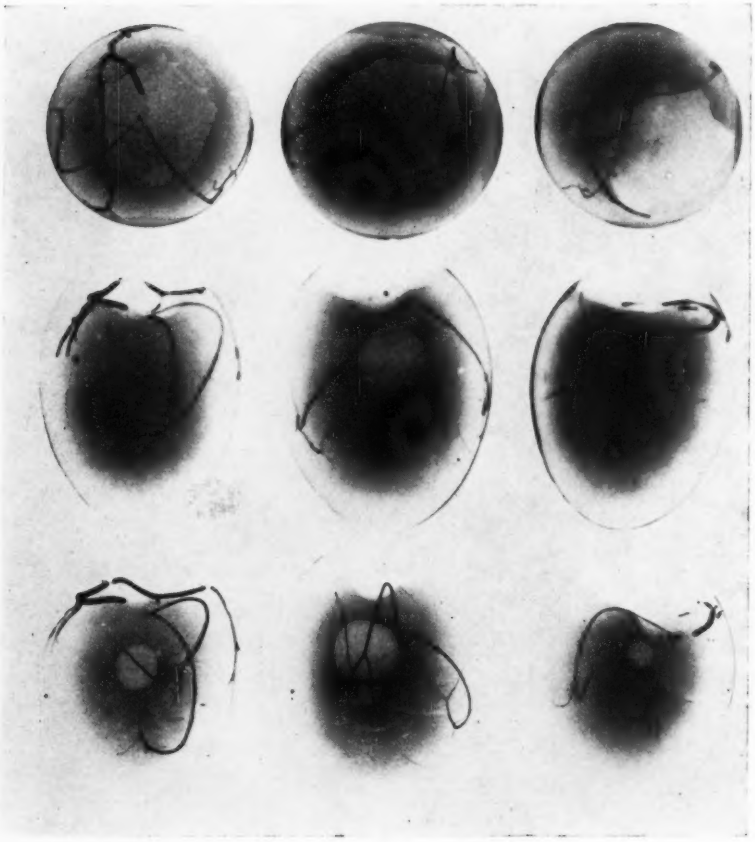
X-ray photographs of three eggs incubated for 10 days at 40 pounds pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after approximately 180° rotation.

PLATE IV



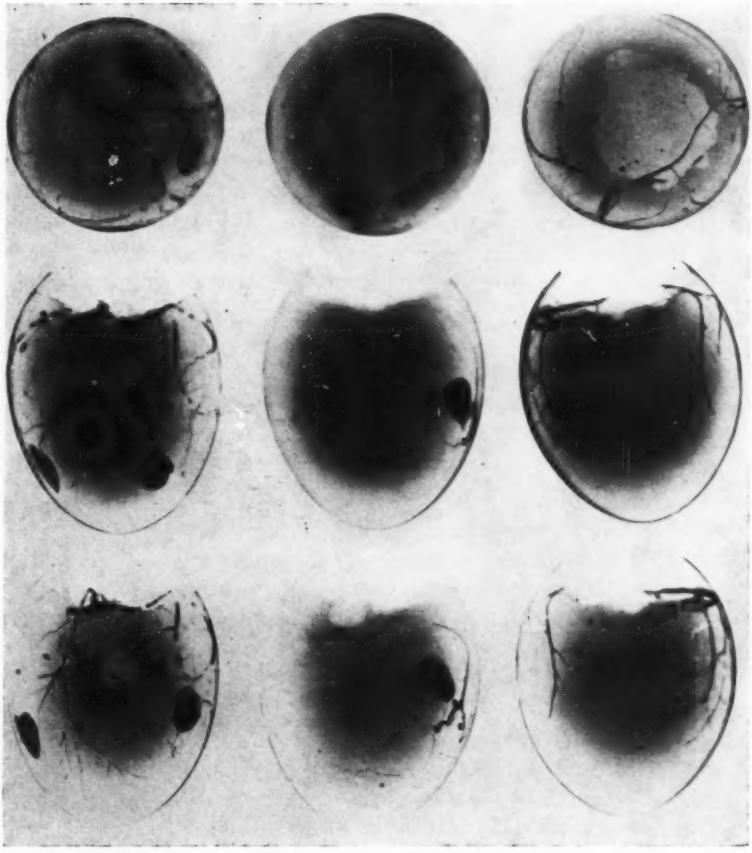
X-ray photographs of three eggs incubated for 10 days at 40 pounds pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after approximately 180° rotation.

PLATE V



X-ray photographs of three eggs incubated for 8 days at atmospheric pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after approximately 180° rotation.

PLATE VI



X-ray photographs of three eggs incubated for 8 days at atmospheric pressure.
Top—air-space end view.
Middle—side view.
Bottom—side view after partial rotation.

DETERMINATION OF POLARITY IN PELVETIA EGGS BY CENTRIFUGING*

By

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(*Received for publication February 2, 1940*)

Pelvetia fastigiata is a marine Brown Alga belonging to the Fucaceae. It grows in abundance in the upper zone of the tide pools in the vicinity of the Hopkins Marine Station. *Pelvetia* used in these experiments was collected at Point Pinos during May, 1938.

Fruiting tips were stored in an electric ice box for not longer than one week, and when transferred to sea water at 15° C. they shed gametes. The egg capsules contain 2 eggs each, instead of 8 as in *Fucus*. *Pelvetia* eggs are larger; the diameters of 100 measured eggs ranged from 82 to 113 microns (av. 103.5), compared with 65-90 microns (av. 75) in *Fucus furcatus*.

This species of *Pelvetia* is hermaphroditic. Fertilization occurs when the egg capsules break down and release the eggs into the sea water, in which free swimming spermatozoa are already present since many of the male capsules dissolve first. The capsule breakdown is readily observed under the microscope. By selecting eggs which have been liberated over a given time-span, it is possible to obtain a population which has been fertilized during the selected time-span.

It has been shown by Knapp (1931) that *Cystosira* eggs form rhizoids at the centrifugal poles after they are centrifuged. Whitaker (1937) has shown that if eggs of *Fucus furcatus* are centrifuged for 15 or 20 minutes at 3000 x g., beginning soon after fertilization, and are then reared in thin cultures to avoid mutual influences ("group effect") in the dark in normal sea water at pH 7.9-8.0, the rhizoids form centrifugally. Ninety-three to ninety-nine per cent form within 10° of the centrifugal pole, which is transparent in the stratified egg. The eggs were embedded at random in firm agar-sea water before centrifuging so that they could not orient in the centrifuge in accordance with any original polarity, and the stratified eggs in blocks of agar were reared in various positions with respect to gravity. Essentially similar results were also obtained in *Fucus* by ultra-centrifuging at 50,000 to 200,000 x g. (Whitaker, 1938).

Similar experiments have been carried out on the eggs of *Pelvetia*. One per cent agar-sea water was poured into ordinary centrifuge tubes

*This work has been supported in part by funds granted by the Rockefeller Foundation.

to a depth of 2 cm., and after this had cooled, upon its flat surface was pipetted a layer of 1% agar-sea water containing *Pelvetia* eggs which had been fertilized 20±5 minutes. This latter layer quickly gelated while the eggs were still in random positions as ice cold sea water was poured on top. The eggs were then centrifuged for 30 minutes at 3500 x g. The upper layer of agar containing the eggs was then removed and cut in blocks which were oriented in sea water so that eggs developed centrifugal sides up, down, and to the side. The eggs were reared in the dark in sea water at pH 8.0-8.2 at 15° C.

The eggs were not crowded in the agar blocks, and results were counted only on eggs at least 10 egg diameters from any other egg, as the "group effect" is strong in *Pelvetia*. Eggs in which the stratified materials had redistributed before the development of the rhizoid also were not counted. The proportion of eggs which redistributed varied greatly with the position in which they were held. Twelve per cent of the eggs with centrifugal poles downward, 41 per cent with centrifugal poles to the side, and 53 per cent with centrifugal poles upward redistributed. In the inverted eggs gravity aids diffusion in bringing about redistribution. The redistribution in *Pelvetia* is greater than in *Fucus* (Whitaker, 1937).

One hundred and fifty-eight eggs with centrifugal poles downward remained stratified. Of these, 90 per cent formed rhizoids on the centrifugal half, 74 per cent within 10° of the centrifugal pole. One hundred and thirty-five eggs with centrifugal poles to the side remained stratified, and 80 per cent formed rhizoids on the centrifugal half (22 per cent within 10° of the centrifugal pole). One hundred and sixteen eggs with centrifugal poles upward remained stratified, and 85 per cent formed rhizoids on the centrifugal half (18 per cent within 10° of the centrifugal pole).

The response of the egg to stratification is thus essentially the same as in *Fucus*, although the polarity is not so precisely determined in *Pelvetia*. This is probably due in part to the greater effect of accumulated metabolites toward the bottom of the dish in the case of this larger egg, and perhaps also to the greater tendency to redistribute. In *Pelvetia* as in *Fucus*, the rhizoid tends to form toward neighboring eggs or toward any impediment to free diffusion of metabolites such as the bottom of the dish.

In 7 additional experiments *Pelvetia* eggs were ultra-centrifuged in small quartz tubes in an air turbine centrifuge at 75,000 x. g., or 150,000 x. g. They were centrifuged for 10 minutes beginning 20±5 minutes after fertilization, and were not embedded in agar. They were reared under the same conditions as before, except that the pH of the

sea water ranged from 7.8-8.2. At these higher forces the eggs were more sharply stratified. Thirteen per cent redistributed. In these experiments, each of which involved 80 to 140 eggs which did not redistribute, the percentage of eggs which formed rhizoids on the centrifugal halves ranged from 83 to 96. The percentage which formed rhizoids within 10° of the centrifugal pole ranged from 35 to 93. The results are thus essentially the same after ultra-centrifuging, although in the best cases higher percentages formed rhizoids within 10° of the centrifugal pole. Fig. 1 shows such a case.

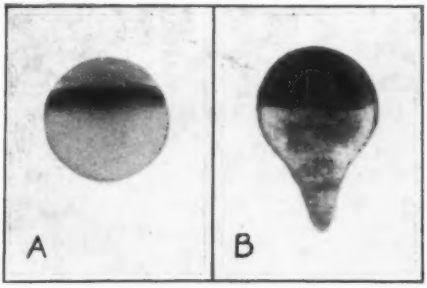

Fig. 1. Photomicrographs of ultra-centrifuged *Pelvetia* egg. (A) very soon after centrifuging and (B) about 40 hours later. The rhizoid has formed at the centrifugal pole.

SUMMARY

1. When *Pelvetia* eggs are centrifuged shortly after fertilization at 3,500, 75,000, or 150,000 x. g., the visible inclusions are thrown centripetally. An oil cap forms at the centripetal pole and plastids etc. aggregate in an adjacent zone.

2. Most of the eggs remain stratified at least to a considerable degree until after the rhizoid forms and the cell divides. If stratified eggs are held with the oil cap downward a larger proportion redistribute.

3. When stratified eggs are reared in the dark in normal sea water (pH 7.8-8.2), separately or in thin cultures to avoid mutual influences, high percentages form rhizoids on the centrifugal halves. This is true regardless of the position with respect to gravity in which the stratified eggs develop. The developmental polarity is thus determined by centrifuging, as in *Fucus* and in *Cystosira*.

4. The polarity is determined by centrifuging when eggs are embedded at random before centrifuging in firm agar-sea water, and the results therefore cannot be attributed to orientation of the eggs in the centrifuge in alignment with an earlier polarity.

5. The eggs of *Pelvetia fastigiata* average about 103 μ in diameter.

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EFFICIENCY OF ENERGY UTILIZATION IN THE GROWTH OF BACTERIA

By

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(*Received for publication February 9, 1940*)

The most efficient food converter among farm animals is probably the pig which retains, during its early youth, 20 to 40% of all physiologically available calories of the food in the increase of body weight. One kg of body weight, with 80% water, corresponds to about 1100 Cal. Therefore, 1 Cal. of food = 0.2 to 0.4 Cal of body weight = 0.182 to 0.364 g live weight = 0.036 to 0.073 g solids. (All data from Armsby and Moulton, 1934).

As example of poikilothermic animals, the trout has been investigated by McCay (1937) and Tunison (1937). The best food conversion was found in the Cortland Hatchery Report No. 6, Table 9, p. 18, where 0.68 g dry beef liver produced 1 g trout. Using Sherman's averages for composition of beef liver, this amounts to 0.068 g trout solids per Calorie of food. Somewhat more efficient is the cockroach which, according to McCay (1938) produces 0.081 to 0.083 g of body solids per Calorie of food.

It seemed interesting to compare with these complex organisms the simple unicellular bacteria and yeasts which are characterized by an enormous rate of metabolism. Streptococci are capable of fermenting twice their own body weight of sugar in one hour, and yeast will ferment 30 to 40% of its body weight in sugar per hour. Fermentation is a continuous process, with no resting periods at night. Such enormous rate might result in waste. Further, the extremely large exposed surface of bacteria might result in a great loss of energy, through excessive radiation or conduction. (The surface of 10^{12} cells of *Bact. coli*, developing in 1 liter of culture, weighing only 220 mg, is about 50 square feet).

Very detailed studies of the energy utilization by the mold *Aspergillus niger* (*Sterigmatocystis nigra*) have been made by Terroine and associates (1922 to 1927). The organism was grown on a mineral solution with glucose as the only carbon and energy source, and NH_3 as the only nitrogen source. 1 Cal. of food produced about 0.125 to 0.145 g of mycelium representing 0.6 to 0.7 Cal. This gross efficiency remained unchanged within the temperature range from 22 to 38°C, and within the pH range from 7.7 to 1.7. It was not affected when the

nitrogen source was changed from NH_3 to urea or guanidine. Nitrates gave almost the same efficiency at pH 1.7 and less; at higher pH, there was a considerable lowering of the conversion factor. The utilization is distinctly better than in animals.

The gross efficiency dropped from 0.58 to 0.39 Cal. when gelatin, peptone, or amino acids were substituted for glucose as only source of energy. It varied between 0.43 and 0.47 Cal. for the various fatty acids. The conclusions drawn by the authors from these measurements are interesting for the biochemistry of growth, but cannot be discussed here.

Quite extensive data on the efficiency of yeast manufacture on a commercial scale have been published by Claassen (1927, 1934, 1935). Other experiments by Claassen, on an experimental scale (7 liter batches) may be found in Rahn (1932, p. 238/9). In a typical example, the yeast consumed 998 kg invert sugar, 152 kg amino acids (from molasses) and ammonia salts; the yeast solids increased 394.1 kg, and 210.6 kg alcohol was formed. The alcohol corresponds to 412.5 kg glucose fermented, producing only 0.1495 Cal. per gram. The remaining sugar, $998 - 412.5 = 585.5$ kg, was oxidized or used as material for cell construction. So were the amino acids. The total Calories of food consumed, minus Calories of fermentation products, amounted to 2,903,700 Calories. They produced 394.1 kg yeast solids or 0.135 g per Calorie. Some of the smaller scale experiments give values as high as 0.24 and 0.26 g yeast per Calorie. This is approximately five times the efficiency of vertebrates, and means 0.6-1.2 Calories of body weight per Calorie of food consumed. It is quite evident that the small size, large surface and rapid rate of metabolism of yeast do not tend to produce waste of energy, but rather increase efficiency of food conversion.

When the organisms become still smaller, this efficiency of food conversion does not appear to be increased. Experiments by Rubner, Tangl and others are extended far beyond the period of rapid growth, and "maintenance" makes the efficiency far too small (Rahn, 1932, p. 183). The energy utilization of rapidly multiplying bacteria can be computed from their oxygen consumption (Rahn, 1940). The combustion heat of practically all organic compounds is 3.3 Calories per gram oxygen. If we know the oxygen demand per cell per hour, and the time required for the doubling of cells, the increase in live weight per Calorie can be computed, and the dry weight is approximately 25% of the live weight. These values are given in the Table.

However, these results must be corrected before they are comparable to the data for yeast or for animals. There, the energy contained in the building material for the new growth was included in the total Calories of the food, while with bacteria, the oxygen gives only the energy ob-

AVERAGES OF DATA ON BACTERIA MULTIPLYING AT 30°C IN PEPTONE SOLUTION

	cell weight in 10^{-10} mg	generation time in minutes	O_2 per cell per hour in 10^{-10} mg	O_2 per generation in 10^{-10} mg	gram live weight produced per gram oxygen	gram live weight produced per Calory of respiration	Cal. of growth per Cal. of respiration
Bacterium coli	7.5	42	3.9	2.7	2.78	0.87	0.98
Bacterium cloacae	7.5	37	4.0	1.9	3.95	1.23	1.38
Bacterium aerogenes	7.5	38	3.3	2.05	3.66	1.14	1.28
Bacterium levans	7.5	43	5.6	4.0	1.85	0.58	0.65
Bacterium communior	7.5	55	3.0	2.6	2.88	0.90	1.00
Bacterium vulgare	7.5	51	5.3	4.5	1.67	0.53	0.60
Pseudomonas fluorescens	7.5	64	9.3	9.1	0.83	0.26	0.29
Pseudomonas aeruginosa	7.5	61	8.4	8.5	0.88	0.27	0.30
Bacillus subtilis	7.5	51	20.6	18.1	0.43	0.13	0.15
Bacillus cereus	15.8	40	32.4	22.3	0.73	0.23	0.26
Bacillus mesentericus	7.5	50	9.5	8.1	0.95	0.30	0.33

tained by respiration. The correction is simple, and is best demonstrated by the concrete example of *Bacterium coli*. 1 Cal. produces 0.87 g live weight of *Bact coli* = 0.218 g solids. These solids were produced from peptone with 5.5 Cal. combustion heat. The total Calories necessary to produce 0.218 g solids were 1 Cal. (from oxidation) + 0.218 × 5.5 Cal. (from building materials). Thus, 2.10 Cal. produced 0.218 g solids or 1 Cal. produced only 0.103 g bacteria solids. The corrections show that the gain per Calorie in the colon group is 0.075 to 0.181 g solids, or 0.3 to 0.72 g live weight. In the *Bacillus* group, and in the *Pseudomonas* group, it approaches a lower level, but remains still in the same range as with mammals.

Bacteria show the same efficiency of food conversion into body substance as the animals. These bacteria were cultivated under normal laboratory conditions, and the experiments were discontinued as soon as the oxygen was exhausted.

It seems probable that the very great efficiency of the bread yeast is the result of long experimentation to obtain optimal conditions, including abundant aeration, and also of a careful selection of the yeast strain. Possibly, similar yields might be obtained with bacteria if there were any commercial inducement in it. The differences in efficiency of Aspergillus obtained with different types of food may possibly apply to bacteria as well, and higher utilization of energy might be obtained in pure carbohydrate media.

SUMMARY

The maximal efficiency of energy utilization in growth is of the same magnitude in the vertebrates, insects, and bacteria studied. The greater efficiency of yeast under the conditions of commercial manufacture may be due to careful selection of the strain as well as to perfect control of optimal conditions.

One Calorie of food produced

in pigs	0.036-0.073 g	solids	= 0.2 -0.4 Cal.
in trout	0.035-0.068 g	"	= 0.18-0.31 "
in cockroaches	0.082-0.083 g	"	= 0.34-0.35 "
in molds	0.125-0.145 g	"	= 0.58-0.70 "
in yeast	0.135-0.250 g	"	= 0.60-1.10 "
in Colon bacteria	0.075-0.181 g	"	= 0.34-0.81 "
in Bacilli	0.028-0.053 g	"	= 0.13-0.24 "
in Pseudomonades	0.048-0.050 g	"	= 0.21-0.22 "

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SYNTHETIC l-LEUCINE IN DEVELOPMENTAL GROWTH OF OBELIA GENICULATA

By

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In 1933 a series of 18 experiments was run with an l-leucine of unknown purity. Because the number of trials was too few and the range of concentration too narrow for satisfactory evaluation, a second series of 60 experiments was run in 1938 (Hammett and Hyde, 1939).

For this there was used a specially prepared product which was claimed to have the purity recorded in the cited report. In that report particular attention was paid to the possibility that the observed enhancement of the proliferation phase of obelia development might have arisen from contamination by some sulfur-bearing compound such as methionine (Mueller, 1935). This idea was rejected at the time because of the analyses given by the manufacturer.

I was uneasy over the matter and sent what was left of the product to Dr. Toennies of The Institute Staff for a sulfur determination. He reported 0.08 per cent sulfur instead of the less than 0.016 per cent given by the manufacturer. Since this amount was adequate, if in proper form, to produce the observed stimulation of cell increase in number, and since correspondence with the manufacturer gave no clean-cut evidence his analytical results were valid, it became evident the matter of proliferation reaction to l-leucine was decidedly up in the air.

In view of the then apparent impossibility of getting a sulfur-free l-leucine from natural sources it was decided to test out a synthetic product.

The l-leucine now used was prepared by resolution of a *synthetic* dl-leucine purchased from the same manufacturer who supplied the *natural* product. It had an optical rotation of $[\alpha]_D^{27} = +16.2 \pm 0.2°$ in hydrochloric acid solution. Comparable values in the literature are $+15.4$; $+15.6$; $+15.7$; $+15.9$ with some higher due to contamination with isoleucine. The titration value was 130. $\pm$ 0.1 as against a theoretical of 131.1. This gives an indicated purity of 100.5 $\pm$ 0.1 per cent. The microscopic picture showed homogeneity.

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With this compound 63 experiments were run with close to 35,000 animals, half of which were exposed to concentrations ranging from M/400,000 to M/12,500. The experimental procedure was as usual. Tests and controls were run simultaneously under like conditions of temperature, illumination, and pH. Four changes into fresh solutions were made during the 24 hour course of each experiment. The results are in Table I which is derived, constructed, and analyzed as described earlier (Hammett and Schlumberger, 1937).

TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN SYNTHETIC l-LEUCINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS AND GONOPHORES AT THE STATED CONCENTRATIONS.

M/1000 x	400		200		100		50		25		12.5	
No. Experiments	10		10		12		10		10		11	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
No. Colonies	149	149	150	150	180	180	150	150	149	149	164	164
No. Hydranths	2785	2789	2766	2774	3315	3318	2736	2720	2665	2672	3126	3123
No. Gonophores	507	515	757	796	272	265	700	656	551	611	275	258
	GROWTH											
	+ − =		+ − =		+ − =		+ − =		+ − =		+ − =	
Initiation	5 1 1		2 2 3		2 3 2		4 1 2		4 2 1		1 3 3	
Proliferation	3 3 2		2 3 3		3 2 3		2 3 3		1 2 5		4 1 3	
Differentiation	6 0 3		3 2 4		2 3 4		4 2 3		3 3 3		1 4 4	
Organization	4 2 2		2 2 3		0 3 1		0 5 3		2 1 5		3 2 4	
	METABOLISM (By Single Experiment)											
Maintenance	2 5 3		3 6 1		2 9 1		4 6 0		5 2 3		8 2 1	
Catabolism	7 3 0		5 5 0		3 9 0		6 4 0		3 6 1		4 7 0	
	METABOLISM (By Experiments Combined)											
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
Regression	24.8	25.2	22.0	22.6	20.2	24.2	25.6	27.2	28.6	26.2	35.7	31.4
Catabolism	74.6	77.8	71.7	71.3	77.7	73.5	66.9	66.9	68.5	67.2	72.5	72.3
	% Increase Regression											
%	1.6		2.7		19.8		6.3		−8.4		−12.0	

RESULTS

Although there is some indication at M/12,500 there is no sure evidence synthetic l-leucine is toxic at any concentration used in this study. Thus—whereas at M/25,000 test growth was greater than control in 1 of the 4 developmental activities and less in none,—at M/12,500 it was less in 2 and greater in 1. Further,—of the 34 possible growth expressions at M/25,000, 10 were greater in test than control, and 8 were less; while at M/12,500 a shift occurred and 9 were greater with 10 less. There were no related shifts in direction of catabolic activity and regression.

While inclusion of the M/12,500 results does not alter the general picture, they will be omitted unless otherwise noted because of suspicion of beginning toxicity.

The analysis will be made from the M/400,000-M/25,000 data.

Initiation was enhanced. Test was greater than control in 17 and less in 9 of the 35 expressions given by the 5 sets of experiments. By concentrations it was greater in 3 and less in none. And when the data are combined it was greater in 4 and less in 1 of the 7 potentialities for activity expression. Breaking this down into its components it appears that initiation of recurrent growth was the major contributor to this enhancement. Thus—test recurrence was greater than control in 10 and less in but 2 of the 15 expressions given by the 5 sets of experiments; while in the remaining 20, test progression was the same as control. Neither anlagen nor regeneration initiation was advanced. New stolon production, however, was better in the test cultures. This extended into the M/12,500 concentration. Test was greater than control in 3 of the 6 sets and less in none. More stolons were produced in 24 and less in 14 of the 63 experiments.

Proliferation expression was essentially unaffected by synthetic l-leucine. What deviation there was tended towards lessened rather than increased activity. Thus—test was less than control in 13 and greater in 11 of the 40 expressions given by the 5 sets of experiments. By concentrations it was greater in none, may have been less in 1, but was essentially equal in all. And when the data are combined it was less in 2 and greater in 2 of the 8 potentialities.

Differentiation was advanced. Test was greater than control in 18 and less in 10 of the 45 expressions given by the 5 sets of experiments. By concentrations it was greater in 2 and less in none. And when the data are combined it was greater in 4 and less in 1 of the 10 potentialities for activity expression.

Organization was held back. Test was less than control in 13 and greater in but 8 of the 45 expressions given by the 5 sets of experiments. By concentrations it was greater in 2, less in 2, and equal in 1. And when the data are combined it was less in 5 and greater in but 2 of the 9 potentialities. This was almost exclusively a hydranth reaction. The gonophores did better than usual. Even into the high M/12,500 concentration.

Indeed, aside from new gonophore production, gonophore growth seems to have been generally improved by synthetic l-leucine. In the proliferation phase, test growth was greater than control in 3 and less in none of the 6 concentrations: it was greater in 6 and less in but 2 of the 12 expressions given by the 6 sets: and it was greater in 44 and less in but 35 of the 126 opportunities given by the 63 experiments.

In differentiation the gonophores like the hydranths were hit by M/12,500 concentration. But in the lower range the trend though slight was towards betterment. Thus—test growth was greater than

control in 4 and less in 3 of the 10 expressions given by the 5 sets of experiments. By concentrations it was greater in 2 and less in 2. And when the data are combined it was greater in 34 and less in 26 of the 104 opportunities given by the 52 experiments.

In the case of gonophore coming to maturity—tests were greater than control in 7 and less in but 2 of the 10 expressions given by the 6 sets of experiments. By concentrations they were greater in 4 and less in 1 of the 6. And when the data are combined gonophore organization was advanced in 27 and retarded in but 9 of the 126 opportunities given by the 63 experiments.

From M/400,000 through M/200,000 to M/100,000 maintenance was increasingly cut and regression increased. At M/50,000 regression decreased and from then on maintenance was increasingly better in tests than controls.

Catabolism reacted irregularly.

DISCUSSION

These experiments were run to see if l-leucine is a proliferation stimulant. They show that *synthetic* l-leucine has no such action. Nor did the natural product used in 1933. Only the product used in 1938 gave this result. But this product, according to Dr. Toennies, carried at least 0.08 per cent sulfur. And leucine is notoriously addicted to contamination with a sulfur-bearing compound methionine (Mueller, 1935). And methionine is a potential source of SH (du Vigneaud, Dyer, and Harmon, 1933). And SH is a specific stimulus of cell increase in number (Hammett, 1929). And methionine enhances the proliferation phase of obelia growth (Hammett and Padis, 1936). And the amount of methionine represented by the sulfur in the 1938 l-leucine was sufficient to yield SH in proliferation stimulating concentration (Hammett and Hyde, 1939).

If then it be granted the synthetic l-leucine used in the present series of experiments is what it is purported to be, only one conclusion is possible. This is that l-leucine as such has no specific stimulating effect on cell increase in number, and that the reaction observed in the 1938 experiments was due to contamination of the l-leucine there used with methionine or some similar sulfur-bearing compound capable of yielding the proliferation stimulus SH in proper concentration.

Correlative evidence is the fact that the slight trend to proliferation retardation observed in 1938 was an acual event in 1933. This is seen in the tabulation of the 1939 paper. The picture is further support—if any were needed—for the SH postulate of cell increase in number.

From the beginning of these studies I have repeatedly stressed the

necessity for absolute purity of compounds used in growth work of this type. The experience with l-leucine is good example of what happens when such restriction is not observed. It demonstrates the need for continual vigilance.

The results reported here immediately throw into doubt ALL growth studies made with leucine which has not been proven free from contamination with a sulfur-bearing compound. Such cannot be considered valid indices of l-leucine participation in growth until repeated with a product which is assuredly free from this powerful growth affecting associate.

The 1938 data can hardly be accepted as expressing the true influence of l-leucine in developmental growth. The distortion of proliferation due to methionine (SH) must have had reverberations in other growth expressions. The proliferation phase is basic to subsequent development. All development is inter-related. The fact that the course of maintenance—which is not a growth activity—reacts the same to sulfur-free synthetic leucine as to sulfur-containing natural leucine; while the expressions of the growth reactions to the two products differ markedly is practical evidence the point is well taken. Consistent is the fact that the 1939 and 1933 growth results show similitude rather than divergence. Thus—in neither is proliferation forwarded: in both differentiation is enhanced: initiation is at least no less intense in test than control and organization is no more.

It will therefore be assumed that the growth reaction to synthetic l-leucine is representative of properties subserved by the natural product.

The trend to better stolon production which obtained here was also present in the 1933 and the 1938 experiments. This consistency indicates that l-leucine is a specific participant in some process essential to that particular growth expression. What this may be is shrouded by ignorance.

The increase in regression with increase in concentration up to the critical point is indication of a specific dynamic action. Its occurrence in the 1938 as well as the 1939 data is strengthening.

There was no essential difference between l-leucine and d-isoleucine with respect to regression influence (Hammett and Toman, 1939). This absence of difference emphasizes the distinction between l-leucine and d-valine in this respect (Hammett and Comee, 1938). For here l-leucine like d-isoleucine produces more regression than d-valine. In other words the addition of CH_2 in homologous series determines an increased regression here as elsewhere. These findings sustain the interpretations expressed in the cited reports.

To be especially noted—but not explained since data therefor are not available—is the fact that the enhancement of regression which is produced by l-leucine in concentrations up to and including M/50,000, is reversed in the higher M/25,000 to M/12,500 range. Here maintenance becomes sustained instead of cut. This happened in both the 1938 and 1939 series and is indicated in the pertinent (50-16.7) group of the 1933.

SUMMARY AND CONCLUSION

Earlier experiments with a sulfur-containing natural l-leucine having yielded proliferation enhancement, it became necessary to determine whether this was indigenous to leucine or attributable to the contaminant. Sixty-three experiments were therefore run with close to 35,000 animals half of which were exposed to a sulfur-free *synthetic* l-leucine in concentrations from M/400,000 to M/12,500. It was found that the amino acid:—

1. Was not overtly toxic at any of the stated concentrations.
2. Was a stimulus to initiation and differentiation.
3. *Had no forwarding influence on proliferation.*
4. Enhanced stolon production.
5. Acted to retard organization expression.
6. Cut maintenance and increased regression in low concentrations: sustained maintenance and decreased regression in high.
7. Had no regular effect on catabolism.

It is concluded that l-leucine as such has no specific effect on proliferation, and that the previous result was due to contamination with some sulfur-bearing compound—presumably methionine—capable of yielding the proliferation stimulating SH.

Supplementary is the fact that l-leucine like all other amino acids and nucleic acid derivatives has its own peculiar and individual influence on obelia developmental growth. In the metabolic field it shows some slight specific dynamic effect, and persistently is more stimulative of regression than d-valine. This gives added evidence for the postulate that the addition of CH_2 in homologous series is determinative of metabolic increase.

The work was done at the Marine Experimental Station of The Lankenau Hospital Research Institute, North Truro, Massachusetts, with aid from Miss Amelia Bein of Philadelphia, and the technical assistance of Misses Steele, Comee, and Hyde and Messers Toman and Rivard.

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l-HISTIDINE IN DEVELOPMENTAL GROWTH

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Six years ago I reported the influence of l-histidine on growth was indirect, through its sustaining effect on maintenance (Hammett, 1935). This conclusion was based on data which, in the light of succeeding work, were too few to allow complete evaluation. A repetition and extension of the study was indicated and has been made.

As usual the colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. Experimentation followed the procedure described in previous reports. Tests and controls were run simultaneously under like conditions of temperature, illumination, and pH. Four changes into fresh solutions were made during the 24 hour course of experiment. Seventy experiments were run with close to 39,000 animals, half of which were exposed to the amino acid in concentrations ranging from M/400,000 to M/6250. The data are given in Table I which is derived, constructed and analyzed as described by Hammett and Schlumberger (1937).

The free base was used instead of the hydrochloride because the former gives a pH of about 7.6 and needs but little buffering in seawater, while the latter requires considerable neutralization and is not well defined in composition. The optical rotation was $[\alpha]_D^{26} = -38.1° \pm 0.2°$ in aqueous solution as compared with literature values of —37.9°; —38.1°; —39.4°; and —39.7°. Perchloric acid titration gave 99.4 ± 0.2% purity.

RESULTS

In these experiments l-histidine showed beginning toxicity at M/50,000. Thus—whereas at M/100,000 test growth was greater than control in 2 and less in 2 of the 4 developmental activities, at M/50,000 it was greater in none, and less in 1. Further—of the 34 possible growth expressions at M/100,000, 9 were greater in test than control, and 10 were less; while at M/50,000, 12 were greater and 16 were less. A shift towards lessening of catabolic expression from 7 plus and 3 minus to 3 plus and 6 minus which was sustained in increasing concentrations is consistent (Hammett, 1936; Hammett and Toman, 1939). Review analysis of the 1934 data shows toxicity present in M/31,000. This was not recognized at the time.

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TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN l-HISTIDINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

M/1000 x	400		200		100		50		25		12.5		6.25	
No. Experiments	10		10		10		10		10		10		10	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
No. Colonies	150	150	150	150	149	149	150	150	150	150	147	147	147	147
No. Hydranths	2787	2786	2949	2957	3062	3055	2864	2868	2663	2664	2587	2585	2633	2635
No. Gonophores	80	131	206	198	209	207	127	96	210	214	216	213	159	187
	GROWTH													
	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Initiation	3	2	2	1	4	2	1	3	3	3	3	1	1	3	3	0	6	1	0	5	2
Proliferation	4	2	2	3	1	4	0	5	3	3	5	0	3	3	2	2	4	2	2	5	1
Differentiation	2	6	2	2	6	2	4	2	3	4	5	1	3	3	4	3	4	3	3	6	1
Organization	5	1	2	4	1	2	4	0	3	2	3	3	3	3	3	1	6	2	1	6	1
	METABOLISM (By Single Experiment)													
Maintenance	3	6	1	5	2	3	9	1	0	8	2	0	4	5	1	4	5	1	3	7	0
Catabolism	3	6	1	5	4	1	7	3	0	3	6	1	3	6	1	4	6	0	1	7	2
	METABOLISM (By Combined Experiment)													
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
Regression	27.5	29.6	30.0	26.6	33.3	23.2	31.1	25.5	27.7	29.1	34.0	33.9	29.7	32.1
Catabolism	72.2	70.3	68.8	70.8	76.0	78.7	75.9	73.0	76.4	74.0	78.0	76.3	84.4	65.4
	% Increase Regression													
%	7.6		−11.4		−30.1		−18.0		5.1		−0.3		8.1	

M/25,000 histidine does not determine much increase in toxicity expression. About the only change is a shift from retarded to accelerated regression of complete hydranths. At M/12,500 however the effect is in full swing, for here test growth was less than control in 3 of the 4 developmental activities and greater in none; while of the 34 growth expressions, test growth was less in 20 and greater in but 6. Adequate increase at M/6250, is evident.

Turning now to growth reaction in the non-toxic M/400,000-M/100,000 range it is seen that the developmental activity of initiation was retarded. Thus—test was less than control in 9 and greater in but 5 of the 21 expressions given by the 3 sets of experiments. By concentrations it was less in 2 and equal in 1. And when the data are combined it was less in 4 and greater in 1 of the 7 potentialities for activity expression. No distinctions occur between the several components of this group with respect to reaction to this amino acid.

Proliferation was essentially unaffected. Thus—test was less than control in 8, greater in 7, and equal in 9 of the 24 expressions given by the 3 sets of experiments. By concentrations it was greater in 2 and less in 1. And when the data are combined it was less in 3 and greater in 4 of the 8 potentialities for activity expression.

Differentiation expression was held back. Thus—test was less than control in 14 and greater in but 8 of the 29 expressions given by the 3 sets of experiments. By concentrations it was less in 2 and greater in 1.

And when the data are combined it was less in 6 and greater in 3 of the 10 potentialities.

Organization, however, was forwarded. Thus—test was greater than control in 13 and less in but 2 of the 22 expressions given by the 3 sets of experiments. By concentrations it was greater in all 3. And when the data are combined it was greater in 5 and less in but 1 of the 9 potentialities for activity expression.

The influence of l-histidine on catabolism tended towards enhancement in non-toxic M/200,000-M/100,000 concentrations. Test was greater than control in 12 and less in 7 of the 20 experiments done in the two sets: it was greater in one, and tended to be in the other of the 2 concentrations under discussion and when all the data are combined the percentage activity was greater at both levels. In the toxic range it reacted as usual by retardation. Thus—from M/50,000 to M/6250 test catabolism was less than control in 25 of the 40 experiments and greater in but 11. By concentrations it was less in all 4, and when the data are combined into a single value it is found to be less by an average of 8.5 per cent.

Maintenance was sustained and regression held back by l-histidine here as in 1934. Thus—test expression of maintenance was greater than control in 17 and less in but 9 of the 30 experiments done in the non-toxic range. By concentrations and combined data it was greater in 2 and less in 1 of the 3 sets. And when the data are combined into one value the average difference is found to be 12.4 per cent. As shown by the table this reaction continued on into the M/50,000 set despite beginning toxicity.

Taking then the regression-inhibiting influence as a whole, test hydranths regressed to lesser extent than control in 25 of the 40 experiments and to greater in 11: they regressed less in 3 of the 4 concentrations, and the average salvage was ±13 per cent.

Added emphasis is given by the shift which occurred in the M/25,000-M/6250 toxic range. Here test regression was *greater* than control in 17 and less in but 11 of the 30 experiments: it was greater in 1 and tended to be greater in 2 of the 3 concentrations: and the average *increase* in per cent regression was 4.3 per cent.

DISCUSSION

A toxic effect of histidine on growth has been reported by others (Wyon and McLeod, 1923; Odiette, 1932; Odiette and Truhaut, 1933; Suzuki and Miyao, 1933). Gordon and McLeod (1926) found it indifferent; but Soltys, Umrath and Umrath (1938) recorded inhibition by high concentrations where low were stimulating.

The results reported here—and with other amino acids—demonstrate that toxicity or non-toxicity is a matter of concentration. It is clear that evaluation in growth of this or any other growth-significant compound depends upon the results of exploration in a surely non-toxic range. To accomplish this trials must be made over a wide range of concentrations, viz.—from the low non-effective, through the medium effective, to the high toxic. Such procedure not only eliminates organismic differences: it also reduces the publication of contradictory reports.

Experiments show that histidine is an essential constituent of the ingesta (Mayer and Schaeffer, 1919; Rose and Cox, 1924-26; Rose and Cook, 1925; Stewart, 1925; Klose, Stokstad, and Almquist, 1938; Rose, 1938 (Review)). However, Mosher, Saunders, Kingery, and Williams (1936) consider it unnecessary for *Trichophyton interdigitale* when other amino acids are given with vitamins.

Since it can replace nitrate in plant nutrition (Skinner, 1912) and can be used by yeast (Edlbacker, Becker, and Segasser, 1938) it is not strange that bacteria and other plant forms are encouraged thereby (Kobayashi and Nishikawa, 1921; Fitting, 1927; Muroo, 1935; Fildes and Richardson, 1935; Farber, 1938; Soltys, Umrath and Umrath, 1938).

This influence is not applicable to all growth expressions. Thus the amino acid has no apparent effect on the straight growth of the *Avena* coleoptile (Avery and Sargent, 1939) nor on the production of 'giant' yeast cells (Holt, 1928).

Furthermore although Conrad and Berg (1937) found that subcutaneously administered histidine supports growth; Weiss, Aron, and Holtzmann (1933) got no help for lesions of the gastric mucosa from intravenous injections.

It is possible to attribute the difference to difference in path of exposure. It is also possible, and personally preferable, to attribute it to difference in growth expression being recorded. In the one case weight was the index: in the other cell multiplication. The weight betterment may be attributed to a sustaining influence of histidine on maintenance (Hammett, 1935). The lack of favoring effect on proliferation also appears in the obelia data.

This specificity of histidine growth effect with respect to expression affected is consistent with the obelia studies where the several aspects are also differently reactive. It is from such that evaluation emerges.

A certain amount of data indicates that erythropoetic tissue activity is stimulated by histidine (Fontes and Thivolle, 1930; Cuthbertson, Fleming, and Stevenson, 1931; Serio, 1931; Aron, 1933). Since this

amino acid is a considerable component of hemoglobin a luxus supply of an essential ingredient is suggested. On the other hand organization expression in obelia is favored. It is therefore possible the erythropoetic reaction is due in some part to stimulation of erythropoetic tissue organization. Consistent is McCarrison's (1924) report that histidine hastens metamorphosis, and Block's (1934) concept that the basic amino acids are primary in the genetic and embryological development of the tissue proteins.

Chemical structure and synthesis; the feeding experiments of Ackroyd and Hopkins (1916), Degan (1938), Crandall and Young (1938) and workers already cited; the analyses of embryological material by Tomita (1929), Calvery (1932) and others; and the critical review of Mitchell and Hamilton (1929) demonstrate the existence of *metabolic* relationship between histidine and the purines. The findings do not, nor can they, because of inadequate design and material, give evidence of an associative relationship between histidine and the purines with respect to *growth*. It remains for the obelia studies to bring this out and set it forth.

It has been found that the purines, adenine and guanine, forward the developmental activity of organization in obelia (Hammett, 1938; 1938a) while xanthine and hypoxanthine exhibit no such action (Hammett and Porter, 1938; Hammett and Steele, 1938).

It is legitimate to attribute the difference in action to difference in chemical make-up.

Now histidine of all the amino acids, and adenine and guanine of all the purines are unique in having both imidazole and NH_2 as molecular components. And these three compounds are unique in being the only naturally occurring tissue components of general distribution which have a generalized and uncontaminated* stimulation effect on organization expression.

It follows then that the property of forwarding organization insofar as histidine, adenine, and guanine are concerned is derived, in all probability from the chemical association of imidazole and NH_2 in the molecule.

Indications for future refinement of the allocation are had. Thus—where both NH and NH_2 are present, there one or another expression of organization is enhanced, not only with imidazole NH, but also with that of guanidine (Hammett and Chatalbash, 1935) and indole (Hammett and Elliott, 1935). In the two latter instances however, the effect is either not general, or it is correlatable with increase in associ-

*An uncontaminated effect is one not clearly correlatable with another reaction.

ated growth expressions, and hence unseparable as a specific reaction of organization.

In the guanine report (Hammett, 1938a) the organization function was tentatively assigned to potentiality for imidine formation in intermediary metabolism. But such cannot be derived from histidine at first sight.

Now nothing is known of the ring-forming capacities of these compounds, or their true spatial configurations and intra-molecular relations. On the other hand the studies of Schoenheimer, Ratner, and Rittenberg (1939) do show that chemical processes take place continuously in living proteins, with alpha nitrogen atoms being transferred from one amino acid to another: with temporary opening of peptide linkages: and with continuous replacement of amide N. Histidine especially not only yields nitrogen, it also accepts it from other amino acids. (Schoenheimer, Rittenberg, and Keston, 1939).

These demonstrations sustain the idea that a common chemical constellation is specifically produced from the NH_2 and imidazole of l-histidine, adenine, and guanine in the basic reaction interchange of living. Whether this be imidine or some allied group is of no immediate importance. What is important is the probability that this common derivative is a determining participant in some process essential to structural organization of obelia to functional effectiveness.

It is through this chemical configuration that an associative relationship between histidine and the purines with respect to growth is demonstrated and established.

The interpretation is independent of the concept that histidine is a precursor of purines. That idea—which is conjectural, not factual—deals with origin and synthesis. Our studies deal with growth function and influence. Exploration of their contacts will be made in a forthcoming correlating monograph.

The outstanding reaction to l-histidine is not of organization but of maintenance. The correlation between regression of the complete hydranths and colonial metabolic activity on the one hand, and regression and metabolic reaction to amino acid on the other has been demonstrated and discussed in preceding reports (Hammett, 1935; 1936; 1936b; 1936c).

Increased regression in non-toxic concentrations tends to parallel specific dynamic effect in higher organisms and is taken as a mark thereof. Conversely, decreased regression is evidence of sparing action and thus of maintenance support.

The consistent appearance of this reaction to l-histidine in the 1936

as well as in the 1934 series, together with the fact that the two sets of observations were obtained with different technical assistants and different preparations sets the influence above any possible question.

Its solidity is enhanced by the fact that it is unique to l-histidine of all the amino acids; the others tending to produce an increase rather than a decrease in regression of the complete feeding animals.

Correlative data are consistent with these findings and support this interpretation.

The conclusion of Rapport and Beard (1928), Miyao (1935) and others, that histidine has no specific dynamic effect, is sustained by the absence of increased regression in non-toxic concentrations. The finding of Birchkov (1936) that the amino acid decreases the respiratory activity of erythrocytes, and of Geiling (1917) that it is essential for maintenance of mice is reflected in the strengthened maintenance of the feeding hydranths. And the implication of Nielsen, Gerber, and Corley (1939) that the compound is peculiarly useful in sparing tissue nitrogen is at one and the same time confirmation and explanation of the retarded regression of the metabolically active hydranths; and substantiation of the designation of histidine as "- - - a specific participant in the regulation of maintenance metabolism." (Hammett, 1935).

This extends and clarifies the 1934 postulate regarding the retardation of mass growth of vertebrates when diets deficient only in histidine are fed, and its resumption when the amino acid is re-administered. It appears that this may be attributed more to a lessened sparing of tissue nitrogen and its restoration—with the parallel maintenance responses—than to any direct participation of the amino acid in processes concerned specifically in anabolism or weight increment growth.

In previous reports this function has been assigned to the imidazole moiety. The evidence can now be solidified.

In the analysis of histidine effect on organization it appeared that stimulation resided in a derivative of the $NH\text{-}NH_2$ combination.

In the case of maintenance support this combination is inactive in amino acids other than histidine. Neither arginine nor tryptophane act thusly. Indeed they cut maintenance and increase regression considerably (Hammett and Chatalbash, 1935; Hammett and Elliott, 1935). NH_2 alone is ineffective, for the other amino acids which carry the group do not support maintenance. And NH cannot be invoked for proline too acts to increase rather than decrease regression (Hammett and Collings, 1938).

These observations suggest the maintenance supporting property

resides in imidazole as a nucleus rather than in any particular component thereof.

The possibility must not be avoided, however, that NH_2 is supportive of imidazole function since this is not had when NH_2 is absent as in xanthine and hypoxanthine (Hammett and Porter, 1938; Hammett and Steele, 1938); but is always had when NH_2 is present, as in histidine, adenine, guanine, and allantoin (Hammett, 1935; 1936; 1938; 1938a). (The student will discriminate between the alleged organization factor NH-NH_2, and the possible maintenance factor imidazole NH_2).

On the other hand imidazole may be ineffective in xanthine and hypoxanthine, not because NH_2 is lacking, but because it is not unlimbered for action in intermediary exchange; or perhaps because it is blocked or neutralized by contiguous groups or by-products.

Evidence is given in the cited reports, that xanthine and hypoxanthine may split to pyrimidine and urea: while adenine and guanine go down via allantoin. If this is so, imidazole integrity is lost in the first case, and preserved in the second. Herein indeed may lie the difference in maintenance influence of these purines.

The fact that adenine and guanine support maintenance as does l-histidine is no proof the latter is precursor of the former. It only indicates the probable factor is a common chemical configuration.

The fact that allantoin acts to retard regression as does l-histidine is no proof histidine is the mother substance of allantoin. It only indicates that a chemical group of common possession is the probable factor of influence.

These distinctions must be kept clear.

Other methods must be used to establish the source relationship. What these data do show is that a group of naturally occurring chemical compounds possessing a common chemical moiety have a common effect which is not shown by compounds not having the configuration; from which the conclusion is derived that it is this particular chemical association which is the effective factor in the observed reaction.

It is inevitable that in colony living the status of any one group has its effect upon that of the others. Hasty thought, knowing that in the life-cycle of an obelia hydranth the structural change of organization passes over after a time into the structural change of regression, and knowing from the foregoing analysis that chemical factors for both these changes are present in the imidazole nucleus, might assume the two reactions are expressions of a common denominator.

On *a priori* grounds, and according to the principle enunciated in

The Nature of Growth (Hammett, 1936d) that each developmental activity has its own set of determinants, participants and reactions, such assumption can be questioned. Actual evidence that such can hardly be is had from the already-noted fact that colonies in *control* cultures under laboratory conditions show positive rather than negative correlation between regression and organization.

It must always be remembered that interpretation of the growth influence of histidine or any other growth-significant substance or condition should take account of—1. The thing growing. 2. Its state of development at time of observation. 3. The growth expression being recorded.

SUMMARY AND CONCLUSION

The 1934 experiments with l-histidine have been repeated and extended with new material and a new crew of assistants. Seventy experiments were run with close to 39,000 animals (*Obelia geniculata*) half of which were exposed to the amino acid in concentrations ranging from M/400,000 to M/6250. It was found that:

1. Initiation and differentiation were retarded.
2. Proliferation was unaffected.
3. Organization was enhanced.
4. Catabolism tended to be slightly increased.
5. As in the first series, maintenance was sustained and regression decreased.
6. Toxic action appeared at M/50,000.

It is concluded that l-histidine, like all other naturally occurring tissue components of general distribution so far evaluated, produces an individual and characterizing effect.

Its maintenance sustaining influence is outstanding: is associated with the imidazole moiety: and is the basis of the recorded favoring effect on mass increment: probably because of a sparing action on tissue nitrogen.

From correlated results with other compounds it is apparent that the organization forwarding influence of l-histidine is associated with the NH and NH_2 components or some derivative thereof. The fact that l-histidine with this constitution has this property is support for the postulate as set forth in a preceding report.

The work was done at The Marine Experimental Station of The Lankenau Hospital Research Institute, North Truro, Massachusetts, with generous aid from Miss Amelia Bein of Philadelphia, and the technical assistance of Misses Steele, Comee, and Hyde, and Messers Rivard and Toman.

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THE INFLUENCE OF EXTRA-ALKALINE SEA-WATER ON THE DEVELOPMENTAL GROWTH OF OBELIA GENICULATA

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It is a matter of elementary scientific knowledge that environmental pH is a conditioning influence of growth. This is perhaps most strikingly observed in plants (Boysen Jensen, 1939). What—if any—particular aspect of growth is specifically affected, however, and how the influence is exerted, are problems as yet to be undertaken. It may be that the pH sensitivity of the redox equilibrium provides one of the avenues of ingress. Provocative is White's (1939) suggestion from tissue cultures of *Nicotiana* callus, "- - - that oxygen gradients may be important factors in controlling differentiation."

During the past ten seasons of work with obelia we have noted that the pH of our sea-water may fluctuate from 8.0 to 8.8 as determined by cresol red and thymol blue. The water is not that of the open sea but of Massachusetts Bay at the Truro hook which is here freely open to the sea and uncontaminated by sewage or manufacturers' waste. While floating plankton may be and probably is a factor in these variations (Haas, 1916), indigenous vegetation is not, for with the going of the eelgrass which took place some years ago, this has become so sparse as to be negligible.

It may then be assumed that these changes which are produced by the character of the littoral as well as those of temperature and salinity are of influence upon the growth process of our experimental animal.

As a first step towards learning the nature of this influence 10 experiments were run with close to 5000 animals, half of which were exposed to sea-water brought to a pH of 9.4 by the addition of 1.67 c.c. N/10 NaOH to every 500 c.c. No insoluble precipitate was produced when the alkali was added drop-by-drop with vigorous stirring, and none appeared on standing. In this respect our experience differs somewhat from that of Haas (1916), perhaps because our sea-water was naturally more alkaline than his, and because it was more free from contaminants than the Woods Hole samples.

As usual the colonial-living form of the marine hydroid *Obelia*

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geniculata was the test animal. The experimental procedure was that described in previous reports. Test and control cultures were run simultaneously under like conditions of temperature and illumination. Four changes into fresh solutions were made during the 24-hour course of experiment.

RESULTS

It is unnecessary to use space in tabulation.

There was no particular evidence of toxicity. Test growth was less than control in 1 of the 4 developmental activities, greater in 1, and equal in 2. It was less in 14, greater in 12, and equal in 8 of the 34 growth expressions which the colonies afford.

Initiation was essentially unaffected. Test growth was less than control in 3, and greater in 4 of the 7 potentialities of this activity. Of the 70 opportunities for new growth expression given by the 10 experiments, 33 were less in tests than controls, 24 were more, 11 were equal, and 2 were unexpressed.

Proliferation also was essentially unaffected. Test growth was less than control in 3, greater in 2, and equal in 3 of the 8 potentialities. Of the 80 opportunities for proliferation expression given by the 10 experiments, 16 were less in tests than controls, 18 were greater, 30 were equal, and 16 were unexpressed.

Differentiation was advanced. Test growth was greater than control in 6, less in 1, and equal in 3 of the 10 potentialities. Of the 100 opportunities for specialization expression given by the 10 experiments test was less than control in 12, greater in 17, equal in 27, and unexpressed in 44.

Organization was held back. Test growth was less than control in 7, greater in none, and equal in 2 of the 9 potentialities. Of the 90 opportunities for activity expression given by the 10 experiments test was less than control in 16, greater in 4, equal in 25, and unexpressed in 45.

Maintenance was cut and regession increased. Test regression was greater than control in 6, less in 3, and equal in 1 of the 10 trials. It was greater than control by 2.5 per cent when all data are combined. And the percentage increase in regression over the control was 10.4.

Catabolism was essentially unaffected. Test dissolution was less than control in 4, greater in 5, and equal in 1 of the 10 experiments. When all the data are combined test expression was greater than control by 1.4 per cent.

DISCUSSION

It has been reported by Loeb (1904); Moore, Roaf, and Whitley, (1905); Whitley (1905); Loeb and Wasteneys (1911) and others that

the growth of marine organisms is forwarded by OH. Loeb mentions obelia specifically in his 1904 paper but gives no details regarding the nature of the growth response.

There is no evidence from our experiments that growth of the colony as a whole is affected one way or another by added hydroxyl. There is evidence, however, that particular developmental activities are specifically influenced. For in the presence of extra-alkaline sea-water differentiation expression is increased over that which obtains in the medium of natural alkalinity, and organization is held back.

It appears then that insofar as obelia is concerned, a growth enhancing influence of OH-ions may be expressed through the developmental activity of differentiation.

It is interesting to note the implications derivable from this correlation and those reported by White (1939) and Loeb and Wasteneys (1911).

White postulates an association between *oxygen* supply and *differentiation* in plant tissue cultures.

Loeb and Wasteneys found an association between *oxidation* processes in developing arbacia eggs and *basicity* of the culture medium.

And we have found an association between *basicity* of culture medium and differentiation of obelia hydranths.

These three sets of observations provide a triad association of

OH—oxidation—differentiation

which is not to be discarded as unsignificant.

The existence of OH-ion influence upon oxidation processes as represented in both respiration and redox equilibrium has long been known. The existence of oxygen relation to differentiation was not known until White's demonstration. And the existence of a favoring influence of a certain OH-ion level on differentiation has but now been uncovered by our data. The probability that these are not fortuitous associations is strong. And although experimental proof is lacking, the chances are good it will be found that the differentiation forwarding influence of basicity is exerted through an OH-ion influence on some type of oxidation expression.

The OH-differentiation association holds for a sea-water pH of 9.4. It does not necessarily hold for other OH-ion levels. In fact it is probably specific for only a certain narrow pH range. This may well differ for different organisms. Nor does it exclude the possibility of other growth enhancements at other extra-alkaline pH levels. Such indeed are probable.

Assuming that each developmental activity has its own chemical

determinants, participants, and reactions (Hammett, 1939; 1940) it follows that each set thereof must have its own optimum pH level at which growth expression is at a maximum. From which it follows that a naturally or artificially induced deviation from the level optimal for one activity, might yield a level within the optimal range for another. Such an event would result in a lesser expression of the first and a greater expression of the second. Perhaps this is what has happened here where at pH 9.4 differentiation expression is enhanced and that of organization is held back.

It is conceivable that by proper adjustment of pH one could evoke at will a seriatim acceleration or retardation of each of the developmental activities expressed by an obelia colony. Perhaps the tidal changes in pH are accessory regulators of the orderly development of this and other littoral organisms.

SUMMARY AND CONCLUSION

Ten experiments were run with close to 5000 obelia, half of which were kept in sea-water of natural alkalinity and half of which were exposed to sea-water brought to a pH of 9.4 by added NaOH.

It was found that the higher OH-ion concentration level acted to produce a greater than usual expression of differentiation, and a lesser than usual expression of organization. The developmental activities of initiation and proliferation were essentially unchanged. Maintenance was cut somewhat and regression increased. Catabolism was unaffected. There was no overt toxicity.

From these and correlated observations there is suggestion a certain pH level of OH-ions may be a contributing factor to differentiation enhancement through an influence on some phase of oxidation expression.

The work was done at the Marine Experimental Station of The Institute with aid from Miss Amelia Bein of Philadelphia, and the technical assistance of Misses Comee, Steele, and Hyde and Mr. Toman.

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BOOK REVIEWS

The Newer Knowledge of Nutrition, by E. V. McCollum, Elsa Orent-Keiles, and Harry G. Day. Fifth edition, rewritten, pp. 701, $4.50. The Macmillan Company, New York, 1939.

This book is written with the object of bringing together all the pertinent information relating to the field of nutrition with especial emphasis on the recent developments. In the words of the authors "an attempt has been made to prepare a concise survey of the field of nutrition which is more extensive and more inclusive than any other now available."

Discussions of the sources, nutritional requirements, chemical nature, function, and the effects of a deficiency of the various nutrients are included. In addition, there are discussions of the dietary properties of foodstuffs, appetite, dietary habits in different parts of the world, diet in relation to teeth, and diet in relation to healthful longevity.

Each topic is covered as extensively as space permits. References to the important early publications and to the latest reports available at the time of publication are evaluated, and possibilities for future work suggested. Certain subjects are treated in more detail than in most nutrition texts. Magnesium is given the same importance as calcium and phosphorus, and considerable attention is paid to the physiological role of sodium, potassium, and the trace elements. A chapter is devoted to the effect of diet on teeth, and dental caries, termed "the most widespread of human diseases", is discussed in detail.

Tables of the nutritional values of the proteins of different classes of foods, and the supplementary relationships between proteins are given in the appendix. Likewise, the distribution of vitamins A, B_1, D, ascorbic acid, and riboflavin in foodstuffs is tabulated. Interesting illustrations of the effects of specific deficiencies are also included.

It is evident that in a book with such a wide scope as this, differences in opinion should arise in the interpretation of experimental evidence. The authors present their views with which all may not agree. They have succeeded in bringing together a wealth of information not previously organized or presented in any text. This edition of the book, which has been entirely rewritten, should be of interest to workers in nutrition or related fields.

Elizabeth Dyar

Problems of Ageing, E. V. Cowdry, Ed. Foreword by Lawrence K. Frank. Introduction by John Dewey. Contributions by Edgar Allen: Female reproductive system, L. F. Barker: Ageing from the viewpoint of the clinician, W. B. Cannon: Ageing of homeostatic mechanisms, A. J. Carlson: The thyroid, pancreatic islets, parathyroids, adrenals, thymus and pituitary, A. E. Cohn: Cardiovascular system and blood, E. V. Cowdry: Ageing of tissue fluids, M. Critchley: Ageing of the nervous system, W. Crocker: Ageing in plants, L. I. Dublin: Longevity in retrospect and prospect, E. T. Engle: Male reproductive system, J. S. Friedenwald: The eye, S. R. Guild: The ear, G. V. Hamilton: Changes in psychosexual phenomena with age, L. O. Howard: Ageing of insects, A. C. Ivy: Digestive system, H. S. Jennings: Senescence and death in protozoa and invertebrates, E. B. Krumbhaare: Lymphatic tissue, C. M. McCay: Chemical aspects of ageing, W. de B. MacNider: Ageing processes considered in relation to tissue susceptibility and resistance, W. R. Miles: Psychological aspects of ageing, J. R. Oliver: Urinary system, T. W. Todd: Ageing of vertebrates; also Skeleton, locomotor system and teeth, F. D. Weidman: Ageing of the skin, C. Wissler: Human cultural levels. Publication of the Josiah Macy Jr. Foundation, The Williams & Wilkins Co., Baltimore. 1939. XXX+758 pp. $10.00.

This book of essays on ageing is closely related and a fitting sequel to the book on arteriosclerosis also edited by Cowdry[1] and sponsored by the Macy Foundation.

The relation between these two books is particularly close if it is recalled that over half the death rate following middle life is directly attributable to arteriosclerosis and to related cardiovascular disease. Indeed, the degree of senescence may perhaps be quantitatively measured by the ageing of the colloids and accumulation of such metabolic products as cholesterol, both of which constitute sclerosis, especially of the cardiovascular system. The ageing of the *milieu intérieur* and therefore the ageing of the whole body with the concomitant diseases of old age is dependent on the efficiency of the cardiovascular system. In view of the central importance of the cardiovascular system in the ageing process, it is surprising that it receives such relatively meager financial support for research.

It has been generally understood since the time of Minot that senescence is inseparable from growth, that each is indeed only another aspect of the other in the life cycle. However, regardless of the inherent importance of senescence, pressure for quick returns on research

[1]Cowdry, E. V., ed., Arteriosclerosis: A review of the problem. New York, the Macmillan Co., 1933.

investments compels research workers to concentrate on the growth aspects of the cycle. The literature on nutrition is confined almost exclusively to the influence of nutrients on growth. This is as it should be in times of scarcity of food and nutritional knowledge. But the situation is rapidly changing. The era of nutritional scarcity is rapidly passing into one of abundance. Moreover, the era of high birth rate, with its associated apparent high mortality, is rapidly passing into one of low birth rate, with an associated apparent lower mortality; that is, with an increased proportion of aged persons in the social structure. These books sponsored by the Macy Foundation perhaps indicate a change in the attitude of society and of research foundations towards an important trend in our social conditions. At any rate the books are timely in calling attention to ageing, the neglected aspect of the developmental cycle.

This book has several unique features. One is an unusual combination of the social and psychological spirit, illustrated by the essays of Dewey, Frank, Barker, Hamilton, with the traditional laboratory spirit, illustrated by Carlson's repeated question, "What are the *facts*?". A second striking feature is its comparative viewpoint. The book includes ageing phenomena in such diverse organisms as plants, protozoa, insects, fishes, amphibia, birds, mammals; ageing of such diverse organs as heart, spleen, salivary glands, esophagus, stomach, pancreas, liver, skeleton, teeth, sex organs; ageing of such diverse functions as nutrition, intelligence, motivations, psychosexual life, homeostatic mechanisms, susceptibility and resistance to infection. A third conspicuous feature is the doubt expressed in almost every chapter concerning the meaning of ageing: 1) is it the result of passage of time as such, or 2) is it the cumulative effect of the impact of environmental factors which together leave the individual, or his organs, or a limiting organ, damaged? From one viewpoint this question is a bit artificial, inasmuch as environment and organism are inseparable. On the other hand, the homeostatic system includes the influence of the organism on the controllable environment, which environment in turn influences the organisms. Thus, for example, it is impossible not to sympathize with Frank's prophylactic suggestion "If just the feeling of being old can lead to a diminution of functional activities, is it possible to delay ageing by continuing life activities and maintaining a feeling of competence for life?" There is no doubt that the rate of ageing, as the rate of growth, can be modified by controlling the environment, sociopsychologic, nutritional, climatic.

It is impossible to review each of the 27 essays written by almost as many authors. This is not a book to be read in one sitting. Each chapter has an appeal of its own. The reviewer was particularly interested in McCay's essay on the chemical, especially the nutritional-

metabolic, aspects, because this is the reviewer's field of work. Likewise, the vital statistician will take special interest in the essay by Dublin; the endrocrinologist in the essays by Allen, Carlson, Engle, Cannon; the psychoanalyist in the essay by Hamilton; and so on for every chapter. The book is a treasury of source material for ageing phenomena; its lack of a satisfying generalization about ageing presents an intellectual challenge.

SAMUEL BRODY

The Comparative Anatomy of the Mammary Gland, by CHARLES W. TURNER. University Cooperative Store, Columbia, Mo., 1939. 373 pp., LIV plates. $3.50.

This book was written by a dairy husbandman by vocation and comparative biologist by avocation. One half of the book describes the mammary apparatus—mostly its growth and development—in dairy cattle; the other half describes the mammary glands of monotremata, marsupians, monodelphians, mice, men, monkeys, and up to elephants and whales. The book will therefore be useful not only to students of dairy husbandry but of mammalian biology in general. It will be particularly and practically useful to students of veterinary anatomy. There is no other book, to the reviewer's knowledge, on the comparative anatomy of the mammary gland.

The 33 chapters of the book are classified under five headings: I. The gross anatomy of the mammary glands of cattle; II. Microscopic anatomy of the udder of cattle; III. The comparative anatomy of the mammary gland; IV. Anatomy of the mammary glands of ungulates; V. The anatomy of the mammary glands of primates. The book has an extensive bibliography and a good index. It also has suggestions for laboratory exercises and review questions, developed in the course of ten years' teaching of graduate students in dairy husbandry.

SAMUEL BRODY